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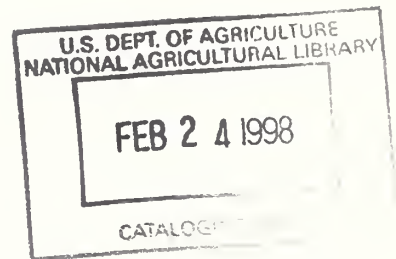
Washington, D.C.

# Proceedings of the Workshop on the Examination of Varieties of Soya Bean

**United States  
Department of  
Agriculture**



**National Agricultural Library**



Workshop on the Examination of  
Varieties of Soya Bean

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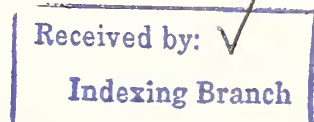
Plant Variety Protection Office  
U.S. Department of Agriculture

with thanks to the

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American Seed Trade Association

University of Maryland





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Workshop on the Examination of  
Varieties of Soya Bean

Program

Wednesday September 27, 1989  
at the Howard Johnson Plaza Hotel

- 8:15 Registration
- 9:30 Welcome and Introductions by the Commissioner of the Plant  
Variety Protection Office  
Dr. Kenneth H. Evans
- 9:40 Introduction and explanation of the program  
Dr. C. Rose Broome, Examiner, U.S. Plant Variety  
Protection Office
- 10:00 FIRST SESSION: Distinctness Under the UPOV Convention
- The Provisions of the UPOV Convention Relating to  
Distinctness and Minimum Distances and Current Suggestions  
for Their Revisions  
Mr. B. Greengrass, Vice Secretary-General, Union  
Internationale pour la Protection des Obtentions  
Vegetales
- 10:30 Coffee break
- 10:50 SECOND SESSION: Variety Examination by the US Plant Variety  
Protection Office
- The US Soya Bean Computer Database: Its Role in  
Determining Distinctness of New Soya Bean Varieties  
Dr. C. Rose Broome, Examiner, U.S. Plant Variety  
Protection Office
- 11:20 THIRD SESSION: The UPOV Approach to Distinctness Testing and  
Minimum Distances
- General Principles of the Testing for Distinctness, as  
seen by a French Examiner  
Ms. F. Blouet, INRA/GEVES, France
- 12:00 Lunch Break

- 14:00      FOURTH SESSION: Special Tests for Soya Bean Varieties
- Current and Future Molecular Tools for Distinguishing  
            Among Soybean Varieties  
            Dr. Reid Palmer, Research Geneticist and Professor,  
            USDA, Agricultural Research Service and Iowa State  
            University
- 14:30      Electrophoretic Determination of Distinctness in Soybean  
            Varieties  
            Dr. Richard C. Payne, Lab Chief, Federal Seed  
            Laboratory, USDA, Agricultural Marketing Service
- 15:00      Coffee Break
- 15:20      FIFTH SESSION: Breeders' Views on Distinctness Testing
- Report of the American Seed Trade Subcommittee on Minimum  
            Distance Determination in Soya Bean  
            Dr. John A. Schillinger, Executive Director of  
            Agronomic Research, Asgrow Seed Co.
- 15:50      Influence of Environment on Selected Plant and Seed Traits  
            in Soybean  
            Dr. Charles E. Caviness, Professor of Soybean Breeding,  
            University of Arkansas
- 16:20      Breeding Methodology and Its Role in Variety Protection  
            Dr. James R. Wilcox, Research Geneticist and Professor,  
            USDA, Agricultural Research Service and Purdue  
            University
- 16:50      Discussion and Question/Answer period
- 18:30      Social Hour  
            Sponsored for the participants by the American Seed  
            Trade Association
- 20:00      Dinner  
            Organized for the participants at the Howard Johnson  
            Plaza Hotel

Thursday September 28, 1989  
at the Howard Johnson Plaza Hotel

8:00 SIXTH SESSION: Soya Bean Breeding in the USA

The Current State of Soybean Breeding in the USA  
Dr. William Kenworthy, Professor of Agronomy,  
University of Maryland

8:30 Morphological Traits Used to Differentiate Soybean  
Varieties

Dr. Randall Nelson, Curator, Northern USDA Soybean  
Collection, U.S. Department of Agriculture,  
Agricultural Research Service

9:30 Buses leave from Howard Johnson Plaza Hotel for Queenstown,  
Maryland

at the University of Maryland  
Wye Institute

11:00 Welcome and Orientation to the Wye Research and Education  
Center

Dr. Russell Brinsfield, Head of the Center, and Mr.  
Lewis Smith, Field Crops & Soils Program Manager

11:20 Field Visits, Demonstrations and Discussions at the Field  
Plots

Introduced by Dr. William J. Kenworthy, Professor of  
Agronomy

13:00 Lunch

Traditional Maryland Barbeque provided by Asgrow Seed  
Company

at the Field Plots of the  
Asgrow Seed Co. Plant Breeding Facility

14:30 Explanation of the Work Done at the Field Plots

Dr. John A. Schillinger and Mr. Bill Rhodes, Asgrow  
Seed Company

15:00 Field Visits, Demonstrations and Discussions at the Field  
Plots

16:30 End of Field Visit and Return to Hotel

Evening free

Friday September 29, 1989

- 9:00 Welcome by Chairperson  
Dr. C. Rose Broome
- 9:15 PVP and Intellectual Property Rights As They Involve  
Transgenic Soybean Varieties  
Mr. Dennis R. Hoerner, Patent Attorney, Monsanto  
Company, St. Louis, Missouri
- 9:45 Panel Discussion on Minimum Distances Between Varieties  
Panel composed of speakers and the Commissioner, U.S.  
Plant Variety Protection Office
- 12:00 Closure of the Workshop

## Distinctness Under the UPOV Convention

### The Provisions of the UPOV Convention Relating to Distinctness and Minimum Distances and Current Suggestions for their Revision

Mr. Barry Greengrass

Vice Secretary-General, UPOV, Geneva

The general question of the amount of difference from existing varieties necessary to justify a grant of plant variety protection for a new variety has always been a difficult topic. It has legal aspects (e.g., what is the intention and the correct interpretation of relevant legal provisions?), but also practical aspects (e.g., can you distinguish in practice between two varieties under differing environments?). The mixed legal and technical nature of the topic and the difference in the application of the underlying principles to differing plant species caused the UPOV Council to propose that the subject be examined in a series of workshops devoted to particular crops in which representatives of the plant breeding industry, the legal profession, and governments could examine the issue in a practical context. Workshops have been held in relation to begonia and

pelargonium, lettuce and the use of new technology for distinctness purposes, and this workshop on soya bean will be followed by one next week in Versailles, France, on maize.

I am sure that in this specialized audience I can assume a fair knowledge of the history and background to Plant Variety Protection. You will know that the industrial (or utility) patent system in most countries regarded plant varieties as unsuitable subjects for patent protection. Amongst the reasons put forward were:

- (a) The fact that plant breeding in most cases did not involve an "inventive step" (or in the US parlance could not fulfill the criterion of "non-obviousness"), and
- (b) that it was not normally possible for a plant breeder to produce an enabling description, that is to say, it was not possible for him to so describe his "invention" that the average skilled plant breeder could repeat the breeding steps so as to create the same plant variety.

Notwithstanding a widespread demand for protection from plant breeders, the world wide patent system was unable to adjust its criteria to meet the needs of plant breeders with one notable exception, that of the Plant Patent Act enacted in the USA in 1932 and now represented by Sections 161 to 164 of 35 USC.

The Plant Patent Act set out to meet the peculiar difficulties which were thought to exist in relation to the protection of plant varieties. It was limited in its application to plants which had been "asexually reproduced" and it established the further criteria of "newness" and "distinctness".



In order to overcome the difficulty of the "enabling description" requirements of the general patent law, Sec 162 of 35 USC provides that the description of plant variety, the subject of a plant patent application, need only be "as complete as is reasonably possible."

The further requirement of the general patent law that an invention must not have been "obvious" to the average man skilled in the particular art and equipped with knowledge of the state of the art at the time that the invention was made was retained in the plant patent law and made applicable to application for plant patents. It has been suggested that "unobviousness", as it relates to plants, appears to depend upon a characteristic being totally different from that found in similar existing varieties or upon the magnitude of the difference between a characteristic and that found in similar existing varieties. In *Yoder Bros v. California-Florida Plant Corp.* in 1976 the non-obviousness requirement was described as the "hardest to apply to plants". The Court thought that it was intended "to ensure that minor improvements will not be granted the protection of a seventeen year monopoly by the State" and that a variety to be patentable must be a "significant improvement".

However, what was happening in practice? President Johnson's Commission on the US Patent System expressed concern because the USPTO seemed not to apply the criterion of non-obviousness to plant patent applications. When the Senate Patent Committee reported on the legislative proposals which were enacted as 35 USC, it commented in relation to the plant patent provisions that it was "immaterial whether in the judgement of those of the Patent Office, the new characteristics

are inferior or superior to those of existing varieties. Experience has shown the absurdity of many views held as to the value of new varieties at the time of their creation."

The US Plant Patent Act accordingly seems to have created a system whereby asexually reproduced plants are protected not if they are merely distinct, but only if they are distinct in a way which represents a significant or major improvement. In practice, it seems to have proved difficult, if not impossible, to determine what constitutes a significant as opposed to a non-significant difference. The "minimum distance" issue had arrived!

The views I have expressed above concerning the provisions concerning non-obviousness in the law and practice of plant patenting are widely held. However, I must place on record that this view that the non-obvious requirement is largely ignored in relation to plant patent applications is not shared by the United States Patent and Trademark Office which has recently stated in a communication to UPOV that "the standard of non-obviousness applied to plant varieties in both plant utility applications and plant patent applications is the same standard as that applied to any utility application in any other technology".

The next major effort to design an effective system of protection for the products of plant breeding programs began when France, in 1957, invited representatives of a group of European states to a conference to discuss the subject of plant variety protection. This conference began a process which ended with the creation of the UPOV Convention in December 1961. This Convention established the criteria of commercial



novelty, distinctness, uniformity and stability as the basis for the grant of protection.

How did the draftsmen of the Convention deal with the question of the degree of distinctness required for the grant of protection?

The experts involved in the drafting of the Convention had asked themselves a number of questions during the drafting process. For example, should there be one or several distinctive characteristics and should account be taken of the "importance" of characteristics? They thought that breeders had in mind the fixing of a characteristic that was new or of a new combination of characteristics within a particular plant. They thought that it was necessary to express two ideas in the convention: the importance of the difference between the new variety and existing varieties, and the need to define this difference with precision. They admitted that the importance of a characteristic was a vague notion, at least to the extent that it was not judged by reference to the use that was to be made of the variety. The question of the relevance of the utility or merit of the variety to a grant of rights was given careful consideration but it was eliminated since it was thought to be subjective, local, and temporary. It was thought to be difficult to apply even at the national level, but much more difficult internationally.

Notwithstanding the imprecision of the notion of importance (particularly once you have eliminated from its potential meanings the idea of merit), the experts involved in the drafting of the Convention supported its adoption since it did not seem proper "to protect a variety which only differed minimally from an existing variety". I

think we begin to perceive in these words distinct echoes of the rationale (insofar as this is correctly expressed in the Yoder case) for the "unobviousness" requirement of the US Plant Patent Act! The experts expressed the view that the importance of a characteristic varies according to the species under consideration. For example, the color of a flower is more important for a rose than for a potato. It would have been more helpful if they had given examples of the relative importance of characteristics within a single species, for this is where the true difficulties arise.

The Convention established in Article 6 as its distinctness requirement (and here I use the language of the 1978 Revision rather than that of the 1961 Convention) that "whatever may be the origin, artificial or natural, of the initial variation from which it has resulted, a variety must be clearly distinguishable by one or more important characteristics from any other variety whose existence is a matter of common knowledge at the time when protection is applied for", and that "the characteristics which permit a variety to be defined and distinguished must be capable of precise recognition and description".

The creators of the UPOV Convention were of the view that in order to apply the criteria of distinctness, uniformity and stability it was necessary to conduct tests by growing varieties alongside each other in biometrically designed trials and this requirement is now reflected in Article 7 of the Convention. I recognize that the practice in the US is different from most other UPOV countries, in that the US does not require specific trials to have been carried out with the variety but accepts information supplied by breeders and does not specify the nature

of the studies used to derive the information. However, it is useful to describe further the more general UPOV approach since this will illustrate some of the general principles involved.

I want, at this stage, to explain how UPOV operates. UPOV is a Union of States which has enacted laws which conform with the substantive requirements spelled out in the UPOV Convention. UPOV is responsible for co-ordinating activity by member States under the Convention. Its governing body is the UPOV Council, comprised of one representative from each member State, and the Council has established a number of sub-committees including an Administrative and Legal Committee and a Technical Committee. The Technical Committee, in turn, supervises the activities of a number of specific Technical Working Parties concerned with particular crop groups.

In order to secure reasonable harmony between States in the testing of varieties, the Council of UPOV has published a document entitled "The General Introduction to the Guidelines for the Conduct of Tests for Distinctness, Homogeneity and Stability of Varieties of Plants," which establishes general principles pursuant to which "Test Guidelines" for individual species have been prepared and published. These individual Test Guidelines are elaborated by the Technical Working Parties which I have referred to earlier. The members of these Working Parties are taxonomic specialists with in-depth knowledge of the species under discussion and their deliberations are supplemented by observations solicited from interested parties in plant breeding, trade, and scientific circles. Great attention is paid in the guidelines to the states of expression of characteristics, to the appropriate testing

approach for qualitative and quantitative characteristics, and to appropriate statistical analysis of the test results. The standard underlying the UPOV member States' approach to distinctness is that it should be possible without risk of error to distinguish between varieties and, if necessary, it should be possible to positively identify a sample of a protected variety. The General Introduction to the Guidelines concludes that two varieties have to be considered distinct if the difference

- has been determined at least in one testing place
- is clear, and
- is consistent.

Many thousands of certificates of Plant Variety Protection have been granted in UPOV member States with virtually no problem arising with the ability to distinguish varieties one from another or to identify plant material as being derived from a particular variety, so that the fundamental technical efficacy of the system in distinguishing one variety from another is virtually unquestioned.

However the general question of the degree of distinctness necessary to support a grant of plant breeders' rights has remained the subject of much debate. Should it be possible, for instance, to take a selection from an existing variety which is clearly distinguishable from that variety and then to secure in relation to it an independent grant of protection? Similarly, should it be possible to protect a mutant from an existing protected variety and then protect the mutant? Does it matter if a new variety, independently developed, differs from an existing protected variety by only an insignificant morphological



characteristic? To choose a more topical example, should it be possible to protect a transformed line which differs only by the expression of a single gene from the variety which was transformed?

Two differing situations arise here. In the one case, an existing variety has been taken and has been minimally modified so as to create a line which is clearly distinct from it. In the other case, two breeders working independently, over, say, ten or more years, have developed varieties which morphologically, at least, resemble each other very closely. Should these two cases be treated differently?

In any event, how do the provisions of the UPOV Convention deal with examples of this kind? I mentioned earlier that there have been few problems in practice, and accordingly very few cases where issues involving distinctness arise have been before the Courts. There is accordingly a dearth of fully argued cases interpreting laws based upon the criteria established in the UPOV Convention. The UPOV Council has considered the meaning of Article 6(i)(a) and their conclusion whilst not totally definitive or authoritative in a juridical sense is persuasive and is reflected in the General Introduction to the Test Guidelines.

A general conclusion is that the word "important" should be interpreted to mean "important for the purposes of distinctness testing" so that at the end of the day little is added by the word "important" to the requirement that varieties should be clearly distinguishable by one or more characteristics from any other variety which is a matter of common knowledge. The word "important" seems in practice to play a similar role to the "non-obviousness" requirement of the plant patent

system. On one hand, everyone seems to agree that it should not be possible to protect minor "cosmetic changes" or the "plagiaristic copy" of an existing variety but, on the other hand, are happy that the UPOV system is flexible enough to protect new varieties which have been independently developed even where these resemble existing varieties closely. In practice, however, it is not possible to distinguish in every instance the one case from the other so that, particularly in the field of ornamental plants, but also in other areas, some plagiaristic breeding developments are likely to be eligible for an independent grant of protection.

My topic is the provisions of the UPOV Convention relating to distinctness and minimum distances. The U.S. law, the Plant Variety Protection Act, whilst conforming with the UPOV Convention, does not slavishly follow its wording. Section 41(a)(1) sets out the distinctness requirement by requiring that an applicant variety must have

"(1) Distinctness in the sense that the variety clearly differs by one or more identifiable morphological, physiological or other characteristics (which may include those evidenced by processing or product characteristics, for example, milling and baking characteristics in case of wheat) as to which a difference in genealogy may contribute evidence, from all prior varieties of public knowledge at the date of determination within the provision of section 42;"

A notable absentee from this text is the word "important" so that for domestic purposes, within the United States of America, you have

been spared the debate concerning the meaning of this word. I find interesting the addition of the words "as to which a difference in genealogy may contribute evidence" since this concept does not appear in other legal systems. I will be interested to learn something of its practical application.

The net effect of the U.S. provisions concerning distinctness would seem, however, notwithstanding differences in language, to be the same as that of Article 6(1)(a) of the UPOV Convention and to closely resemble the effect of the Plant Patent Act so as to afford protection to most, if not all, varieties (including some that may result from short-cut approaches) that are clearly distinguishable from existing varieties.

The purpose of the UPOV workshops is to examine the application of the current provisions of the UPOV Convention and corresponding national laws to the practical circumstances of a particular crop, in this case soybeans.

It would be amiss of me not to mention, however, discussions now underway concerning possible amendment of the UPOV Convention. The Convention has been in existence now for some 28 years and the accumulated experience of the member States has led to a number of suggestions for its improvement. It will not surprise you to learn that proposals for amendments include a proposal to drop the word "important" from the distinctness requirement. Alongside this change, the proposals introduce in Article 5(3) the entirely new concept, for plant breeders' rights law, of dependence. This Article provides that if a variety is "essentially derived" from a [single] protected variety, the owner of

the right in the protected variety shall have certain rights over the derived variety. The precise nature of these rights is as yet undecided but could include a right to deny the exploitation of the derived variety to its breeder or an entitlement to equitable remuneration in respect of commercial exploitation of the derived variety.

In order for this provision to come into operation the derived variety must first be clearly distinguishable from the variety from which it is derived. It is envisaged that such a derived variety will be essentially derived when it retains almost the totality of the genotype of the variety from which it is derived and, perhaps, when it is obtained using a plant improvement method whose objective is the retention of almost the totality of the genotype of the variety from which it is derived. No variety bred according to a system in which selection within progeny is a major element would become the subject of dependence.

The net effect of the introduction of dependence would be to reduce the attractions of plagiaristic, short-cut breeding approaches which can in some instances result from the application of the UPOV distinctness criterion. On the basis of the latest draft of the UPOV Convention revision proposals, the distinctness criteria will be satisfied whenever a variety is clearly distinguishable from any other variety whose existence is a matter of common knowledge at the time of filing the application. This is a comparatively straight forward finding of fact. Examiners are well equipped to decide whether a difference is sufficient to enable varieties to be reliably distinguished and identified in practice. Under the provisions of a revised Convention, the issues of



originality, plagiarism, the appropriate perimeter of protection to provide an incentive to breeders will be primarily dealt with by dependence.

After the introduction of dependence, under the UPOV system, if two breeders working independently breed varieties which resemble each other closely but which are nonetheless clearly distinguishable from each other, both varieties will be independently eligible for protection. Most plant breeders regard this as a fair and equitable situation.

The UPOV concept of dependence should be distinguished from a similar concept which exists in the patent system. One patent is dependent upon another if it falls within the claims, the words which define the scope of protection, of an earlier patent. A plant variety will be dependent upon another when it retains almost the whole of the genotype, the physical genetic reality, of the first variety.

The revision of the UPOV Convention will take place in the 1990-91 period at the earliest. Once revised, the national laws of UPOV member States must be revised to conform with the Revised Convention. Some time will elapse before dependence is a reality and meanwhile plant breeders and plant variety protection must operate within the existing rules. Even after the introduction of dependence there will still be a minimum distance requirement in order to establish that a variety can be reliably distinguished from others. This is a minimum distance workshop, so let's get to work.

The U.S. Soybean Computer Database:  
Its Role in Determining Distinctness  
of New Soybean Varieties

C. Rose Broome

Plant Variety Examiner  
Plant Variety Protection Office  
USDA, Agricultural Marketing Service  
Beltsville, MD

The Computer System of the U.S. Plant Variety Protection Office

The examination of a new soybean cultivar for novelty (distinctness), uniformity, and stability (or "DUS") in the Plant Variety Protection Office (PVPO) of the United States of America relies on a database of information on all soybean cultivars known to exist and be available to the public. This database is maintained on a multi-user Alpha Micro super-microcomputer in the PVPO, which is located in the National Agricultural Library building in Beltsville, Maryland. The computer system is not a part of the library's information base, but is maintained entirely by staff of the PVPO. The system, which utilizes the STAR™ database management software by Cuadra Associates, Inc., contains about 100 distinct crop databases. One of the largest is the

soybean database, "PVSOY," with 1382 records. Each record contains information on a single cultivar.

The database for each crop kind is created and maintained by the examiner who is responsible for that crop. It contains descriptive information about all cultivars in the crop for which information is available, whether they be publicly or privately developed, or whether or not they are protected under the Plant Variety Protection Act.

#### Source of Cultivar Information

Database records come from several sources. In the United States, unlike other UPOV countries, a cultivar being examined for its PVP eligibility must be compared to all other cultivars of public knowledge that are available to the public. There is no mandatory registration system in this country, and no requirement that a cultivar must undergo government-conducted test procedures in order to be sold as a new variety. There is not even a requirement that a cultivar must be adequately described in the scientific literature before it is released.

As a result of this, the PVPO examiner is obliged to search published sources of variety names and descriptions wherever they may be found. If we are fortunate, as in the case when new cultivar release notices in the major agronomic journals such as Crop Science contain detailed cultivar descriptions, we are able to prepare a reasonably complete variety description and enter it into our database. Often, however, we must build up an adequate variety description piecemeal, from several sources. These include release notices, regional variety trials, and so forth. In the case of many horticultural crops, we must

obtain what data we can from advertisements, such as seed catalog descriptions, in the absence of any other published data from the developer.

#### Requirements for a Certificate of Protection in the U.S.A.

Obtaining a certificate of PVP is optional, but there is powerful economic incentive to do so. In order to receive a certificate, a developer of a new variety must file a standardized application form, plus supporting exhibits that document the distinctness, uniformity, and stability of the new cultivar. All this documentation, plus a 2500 seed sample, a \$250 filing fee and a \$1900 search fee, must be submitted to the PVPO within one year of commercialization or formal release of the variety.

#### Content of the Database Record for a Soybean Cultivar

The format of the database record is dictated by the so-called "Exhibit C" form, or botanical description, which is a vital part of the documentation for the distinctness criterion. The 3-page Exhibit C, or "Objective Description of Variety," lists the major phenotypic characteristics that are known to be useful in distinguishing between varieties in the soybean crop. Data for each characteristic of the new variety is reported by the applicant in a structured fashion, and is frequently coded into two to several character-states. The applicant is responsible for the correctness and validity of his or her data. If data are incomplete or incorrectly entered on the form, then any PVP certificate he may be awarded can be compromised to the point that the

owner's rights will not be protectable from infringement by others. The PVPO does not conduct variety trials, but it expects that the applicant will do so, using appropriate and scientific methodology, and will report accurate, properly analyzed data collected from sufficient locations and years to establish DUS of the variety.

Characteristics included on the Exhibit C form fall into the categories of (1) gross morphology, color, and texture of seed, seedling, and plant, (2) morphometric characters such as seed weight and plant height, (3) biochemical determination of seed and seedcoat protein types, and (4) physiological characters, such as maturity and reaction to pathogens and pests. Except for the continuously variable morphometric characters, they are coded into two or more discrete states. Comparison or "check" varieties are listed for several of the characters.

The database record associated with an "application" variety is comprised solely of the data submitted by the applicant on Exhibit C. Records of non-protected varieties contain documentation as to the published source of the information.

#### Use of the Database in Examining a New Soybean Cultivar

Once the data are compiled on all known and available varieties, the examiner is ready to search the database to verify the distinctness of a newly submitted cultivar.

The applicant is required to state what variety or group of varieties is most similar to the new variety in overall characteristics. It is critical that the applicant choose a "most similar variety" which



indeed most closely resembles his variety in most important phenotypic characters, and that he clearly differentiate the two. This statement of novelty will become the most important documentation of taxonomic distinctness for the cultivar, and will be published in the Official Journal of the PVPO. The applicant's selection of "most similar variety" is always scrutinized by the examiner, who compares in detail the database records of that variety and the new one.

However, the examiner must also verify the distinctness of the new variety from all other varieties in the crop by comparing each of its Exhibit C characteristics with all varietal records in the database. This type of comparison could hardly be accomplished without the aid of the computer.

The STAR™ retrieval software allows for rapid Boolean searches on as many characters as are required to establish distinctness. There are currently 1382 variety records in PVSoy, and a typical complex search with about 15 search terms (characters), such as the one below, takes about 15 seconds to execute.

Below I have reproduced a search as it appears on the terminal screen just after execution. The first column simply numbers the line containing a portion of the search strategy. The "Count" column holds the total number of variety records that satisfy the search expression specified on that line. Since the 12 expressions below are connected by the Boolean "AND", the numbers in the "Count" column become progressively smaller as more records are eliminated by the addition of characters. The numbers (preceded by a marker) appearing beneath each search line are the intermediate record count for records in the entire

database that satisfy the individual character beneath which the number appears.

Srch#	Count	Search expression	Chars. of new variety
S1	852	INSUF NOT 1 AND NOSED NOT 3 '292 '274	Adequate description and a seed source
S2	847	S1 AND SCL=(YEL OR "") '852 '1230 '61	Seed color = yellow
S3	105	S2 AND SSZ=(03:13 OR "") '847 '170 '143	Seed weight = 8 g/100
S4	24	S3 AND HCL=(BUF OR "") '105 '219 '79	Hilum = buff
S5	23	S4 AND CCL=(YEL OR "") '24 '985 '375	Cotyledons = yellow
S6	9	S5 AND HC=(LPR OR "") '23 '99 '891	Hypocotyl = light purple
S7	4	S6 AND SP=(LN OR "") '9 '42 '484	Leaflet shape = lanceolate
S8	4	S7 AND FCL NOT WHI '4 '436	Flower color = purple
S9	4	S8 AND PBC NOT (BRN OR TWN) '4 '456 '183	Pubescence = grey
S10	2	S9 AND PHB NOT (IND OR SDT OR INT) '4 '664 '14 '93	Plant habit = determinate
S12	1	S11 AND MAT=(IV OR V OR VI) '2 '181 '95 '103	Maturity group = V

The fourth column was added by the author to indicate the actual states of the characters in a sample variety that is being examined for distinctness. Note that for qualitative characters such as seed color, the software is instructed to search for a match (SCL=YEL), but is also instructed not to eliminate a record which lacks a value for seed color (as indicated by the "null" symbol, a pair of double quotes).

Quantitative characters such as seed weight are searched within a likely range about the actual mean for the variety, in order to allow for possible environmental effects on such characters in similar varieties (SSZ=03:13). Again, the search contains the null condition to allow for missing data.

Searches of the database include important characters useful in distinguishing varieties. Characters that are broken into discrete categories, or states (e.g. flowers purple or flowers white) are searched using the Boolean "NOT" operator. If, for example, there are only two states of the character "Flower Color," - purple and white, the search term used as appears in line S8 above, which is translated "flower color not white." This retrieves not only all those varieties with the code "PUR," but also any other stray codes or typographic errors that may have crept into the data. Thus this mode of searching also assists in revealing defects in the data, and helps ensure that no varieties are overlooked that ought to be evaluated.

The third search line demonstrates searching of a continuously variable character, seed weight. The average seed weight of the variety under examination is 17 grams per 100 seed. To allow for variation of the character under different cultural conditions, I have searched a range of seed weights, from 13 to 21 g/100. Again, the absence of a value for seed weight does not exclude the variety from further consideration. Seed weight is a starred character for application varieties, so we usually have a value for those, but not necessarily for literature records.

Likewise, for hilum color, we look for a match on the value "BLAck" but we also include the null state. And so on through all 15 search sets, which have been "ANDed" together. Note the intermediate postings provided beneath each character-state, and the final count to the left of all records meeting all criteria entered to that point.



Finally, the number of varieties that are found to be similar in these 14 important characters to the new variety are narrowed down to eight. From that point, the determination of novelty centers on finding clear differences between the application variety and each of the eight varieties whose records were retrieved in the search.

Sometimes examination of the full database record for each variety will reveal other significant characters, not originally used in the search, by which the variety differs. If so, these are noted in the final report written by the examiner.

If no significant difference is found between the application variety and one or more of the varieties retrieved in the search, the examiner will contact the developer of the variety and explain. It is the applicant's responsibility, then, to provide the PVPO with at least one clear difference between his variety and the apparently identical one.

Sometimes the applicant is unfamiliar with the variety retrieved in the search, and must obtain authentic seed of that variety and grow it in trials with his own variety to see if novelty of his variety can be supported. If so, the applicant's data are evaluated and if accepted by the examiner, the examination is concluded favorably to the applicant, and the examiner recommends to the Commissioner that a certificate be issued. If no clear difference can be demonstrated, the applicant is given the option to withdraw the application voluntarily, and if he declines to do so, the examiner will recommend that it be denied.

This concludes the examiner's role in the process. The Commissioner must then sign off on the search and, in the case of a

positive outcome to the search procedure, he will recommend issuance to the Secretary of Agriculture (a formality). The final fee of \$250 is requested, the certificate documents are prepared, and the certificate is issued.

The crop databases maintained by the PVPO are central to the examination process, and are constantly growing and being updated by the examiners. They are valuable not only in the role of an office tool, but as a repository of information about diversity in the major agricultural crop species. The PVPO welcomes voluntary submissions of cultivar information, and urges breeders to submit descriptions of their proven varieties, whether or not PVP protection will be sought for the variety. This will help to ensure that only truly new and distinct varieties will be awarded certificates of protection, and will benefit the entire seed industry.

General Principles of the Testing for Distinctness,  
as Seen by a French Examiner  
(Summary)

Francois Blouet

INRA/GEVES, France

Testing for distinctness of soya bean varieties in France involves a two year growing period in the field with observation of plots in two different locations. The trial lay-out consists of blocks with three replications of ten rows of 25 seeds for the candidate varieties, and three replications of five rows of 25 seeds for the reference varieties.

The distinctness procedure starts with a grouping of varieties based upon the description made by the applicant for your characteristics:

UPOV	N°	9	-	Flower	:	color
	N°	5	-	Plant	:	color of hairs
	N°	14	-	Seed	:	hilum color
	N°	16	-	Plant	:	time of maturity

Within each group, distinctness is assessed by describing new varieties following the UPOV guidelines and list of characteristics (16 in total). The reference collection includes the lists of registered and protected varieties in Northern European countries (mainly France and Germany), plus a few American well-known varieties. The varieties in trials only cover a small part of the UPOV maturity range. The latest ones correspond to the maturity of AMSOY 71, that is to say "early to medium" in the UPOV scale.

During the testing period, a close contact is kept between the official people and the breeder. At the end of the first year of observation, a report is sent to the breeder pointing out the similarities detected between his candidates and the reference varieties. The breeder is invited to provide information on the distinctness of his varieties.

The establishment of distinctness requires the checking of a clear difference on one characteristic or a group of small differences on several characteristics. Minimum distances are either linked to the UPOV description states (a difference of 2 points in a 1 to 9 description scale) or can be quantitatively fixed (3 days for "time of maturity" for example).

They should be verified in at least two different trials. The work made by the technical people from the official testing station is validated by a commission of experts belonging to different professional and public organizations (private and public breeders, growers, seed production controllers, ...) who come and visit the trials.

Electrophoresis is not officially used in D.U.S. procedures but is applied to specific cases as a tool to help decisions. France is willing to introduce it officially but this will require a better knowledge of the genetic determinism to be able to fix minimum distances.

Current and Future Molecular Tools for  
Distinguishing Among Soybean Varieties

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Cultivar identification and techniques to assess cultivar purity are the foundation of commercial seed production and crop certification. Current identification techniques use a combination of field-plot evaluation and laboratory tests. The development and release of numerous soybean [Glycine max (L.) Merr.] cultivars have made traditional methods of cultivar identification inadequate.

Modern chemical and molecular techniques that are used in genetic and biochemical research may have application for genetic purity determination and for cultivar identification. The objectives of this presentation are : 1) to describe these techniques, and 2) to present

their advantages and disadvantages. My purpose is to present four techniques that have potential application for cultivar identification in soybean.

For any technique, traditional or modern, to be used, it must meet certain criteria:

1. quick, rapid analyses
2. repeatable, reliable
3. simple, uncomplicated
4. inexpensive
5. under genetic control (inheritance patterns known)

#### I. Electrophoresis:

Electrophoresis is the movement of particles in an electrical field. This is a commonly used technique for analysis of mixtures of molecules in solution according to their electrophoretic mobilities. Enzymes and other proteins are routinely separated on the basis of various molecular forms by use of electrophoresis.

Isoenzymes are multiple molecular forms of an enzyme sharing a catalytic activity, derived from specific tissues of a single organism. Isozyme gel electrophoresis is a procedure that differentially migrates polymorphic enzyme proteins through a gelatinous matrix in an electrical field. This procedure detects endogenous genetic variation.

Factors to consider in isozyme electrophoresis include:

1. matrix system (starch or polyacrylamide)
2. buffer systems
3. extraction procedures



4. enzyme stains
5. plant tissue
6. developmental stages

In soybean, two publications describe the procedures for electrophoresis (Bult et al. 1989; Rennie et al. 1989). Electrophoresis has been used most extensively for genetic studies (see Palmer and Kiang, 1989, for a review). Earlier use of soybean protein electrophoresis demonstrated its utility in cultivar identification (Larson 1967; Wagner and McDonald 1982; Cardy and Beversdorf 1984). Currently only seed coat peroxidase and trypsin inhibitor protein types are listed on the Plant Variety Protection (PVP) forms.

Using 11 enzyme systems, Cardy and Beversdorf (1984) identified 134 of 174 soybean cultivars. With the addition of three morphological traits, 165 of 174 cultivars were individually identified (Table 1). Isozymes were not successful in separating Harosoy from Harosoy 63 or Clark from Clark 63, but did distinguish Corsoy from Corsoy 79 (Table 2).

In a telephone survey of 10 large and medium-sized soybean companies, six were using seed coat peroxidase and only three were using electrophoresis (Table 3). However, in the next five years, all 10 companies indicated that electrophoresis would be done on soybean irrespective of any future PVP policy. The companies would use electrophoresis to check seed lot purity, possibly to monitor introgression of genes or chromosome segments into breeding populations, and for security (legal) concerns.



The advantages of electrophoresis are:

1. The co-expression (co-dominance) of many enzymes/proteins permits identification of heterozygotes. Thus, fewer seeds need to be tested.
2. The banding patterns are not influenced by environmental interactions.
3. The gels usually display discrete banding patterns. This permits ease of scoring and lessens ambiguity.
4. Results can be obtained within one to several days depending upon plant tissue sampled.

Disadvantages of electrophoresis are:

1. A higher level of technology is required to do electrophoresis than field evaluations.
2. The methods and nomenclature may not be identical among laboratories.
3. Depending upon the crop and breeding method employed, electrophoretic heterogeneity may be present in the seed sample.
4. The number of samples that can be analyzed per unit time is fewer than with field evaluations.

## II. Restriction fragment length polymorphism (RFLP):

An RFLP occurs when the DNA of two related isolates is digested with a restriction enzyme and a difference between the electrophoretic mobility of homologous fragments is visualized. This difference could be due either to base-pair changes at the digestion site of the

restriction enzyme or to internal deletion/isolation events within the fragment. The restriction enzymes (endonucleases) recognize short, specific DNA sequences of four to eight nucleotides. The DNA is cleaved at certain locations into a large number of fragments. To actually use the differences in DNA fragment length, the fragments are first separated by size electrophoretically in agarose gels. The fragments move at different speeds, depending upon their length, with the smallest fragments moving the greatest distance. The visualization process takes place in three steps.

1. The separated DNA fragments are removed from the agarose gel. The transfer process is called Southern blotting. The fragments are absorbed onto a solid membrane support (nylon or nitrocellulose filter). This membrane and the attached DNA is called a 'blot'.
2. A radioactively labeled DNA probe is used to visualize specific sequences of DNA. The blot is exposed to different individual probes. Whenever there is a DNA fragment that has a complementary sequence to the probe's sequence, hybridization of homologous DNA fragments takes place. The blot is washed to remove all probe DNA that has not hybridized with a complementary DNA fragment.
3. The visualization process is done by making an autoradiograph. The blots are dried, and X-ray film is placed in contact with the blots containing the radioactively labeled DNA hybrids. The number and positions of the fragments that hybridize to the radioactively labeled probe will appear as discrete bands.

This visual image of an organism's DNA can be used as a 'fingerprint'. Any difference in the relative positions of restriction enzyme sites between two individuals in a particular region of DNA will be visualized as a polymorphism on an autoradiograph.

The procedures for soybean RFLP technology are available (Apuya et al. 1988; Keim et al. 1989). Figure 1 shows the distance between clusters for a study conducted for Pioneer Hi-Bred, with 10 of 38 lines identified. These 10 lines are important ancestors of modern-day soybean cultivars (Delannay et al. 1983; Specht and Williams 1983). The percentage contribution of ancestral strains to the 'collective' genome of 136 soybean cultivars of hybrid origin of Maturity Groups 00 to IV is given in Table 4. A comparison of Figure 1 and Table 4 indicates that many of the important ancestral lines are closely related.

The advantages of RFLP's are:

1. The co-expression (co-dominance) permits identification of heterozyges.
2. The banding patterns are not influenced by environmental interactions.
3. Any plant tissue can be used as a DNA source.
4. Evaluation is nondestructive and requires only small samples.
5. Availability of an almost infinite number of combinations of the restriction enzyme and probe.

Disadvantages of RFLP's are:

1. A high level of technology is required in most steps of the procedure.
2. A long time is needed to complete analyses.
3. The expense is quite high when compared with electrophoresis.

III. Reversed-phase high performance liquid chromatography (RP-HPLC).

Usually HPLC separates proteins on the basis of hydrophobicity, which is differential solubility in an aqueous versus an organic solvent. This technique employs a column packed with specially coated powder. The mixture to be separated is maintained in the liquid phase. The mixture is applied to the column, and the initial conditions separate compounds with a very high affinity for the packing material. A solvent is passed continuously through the column resulting in a gradual change in solvent composition. As the solvent composition changes, different compounds in the sample become soluble. These compounds continue to move through the column and elute at different times. The length of time that each compound takes to be eluted from the column is called retention time. The compounds are monitored spectrophotometrically and recorded. Quantification of individual compounds is easily determined.

The use of HPLC to separate proteins is widely used in cereal chemistry. Recent advances in the use of HPLC to distinguish among soybean cultivars shows promise for future application (Buehler et al. 1989a, b). Their data showed that quantitative data based on peak area percentage were useful criteria for distinguishing closely related lines

(Table 5). No qualitative differences were found among the 14 cultivars sampled. Retention time ratios for selected peaks were not significantly different among cultivars.

The advantages of HPLC are:

1. Environmental variation is usually negligible.
2. Data can be readily quantified and stored and are amenable to computer analysis.
3. Results can be obtained within several hours.
4. This technique is amenable to automation.

Disadvantages to HPLC are:

1. A high level of technology is required.
2. Acceptable internal standards are not available for many crops.
3. The expense is quite high compared with electrophoresis.

#### IV. Two-dimensional electrophoresis:

Two-dimensional electrophoresis separates protein samples in one direction based upon charge followed by separation of samples in another direction based upon size. This type of electrophoresis has been used in cereals and, when combined with isoelectric focusing, has been valuable in separating seed storage proteins.

Research with two-dimensional electrophoresis of soybean involved about 300 lines. These lines included modern cultivars, progenitors of modern cultivars, genetic stocks, and breeding lines. Most gels depicted 400 or more spots, but further interpretation awaits computer-image analysis (R. L. Nelson, USDA, ARS, Urbana, Illinois, unpublished data).



The advantages of two-dimensional electrophoresis are:

1. Large numbers of proteins can be visualized per sample.
2. The technique is amenable to automation.

Disadvantages of two-dimensional electrophoresis are:

1. A high level of technology is required.
2. Computer image capability not developed.

#### Future Prospects

Electrophoresis will become more widely used in soybean cultivar identification. In maize, about 45 isozyme loci show polymorphisms while in soybean, about 18 loci show polymorphisms. Part of this difference can be attributed to maize being a cross-pollinated plant and soybean being a self-pollinated plant. In addition, very few researchers in soybean are actively seeking 'new' enzyme systems.

Restriction fragment length polymorphisms have been detected between the cultivated soybean and the wild soybean and show about 40% polymorphisms. Within the cultivated soybean only 10 to 15% polymorphisms are evident.

Reversed-phase high performance liquid chromatography has been used extensively in cereals. Initial research in soybean was concerned with the optimization of elution and extinction conditions for separation of seed proteins. RP-HPLC has potential in soybean cultivar identification, but considerably more work is necessary before it will be used on a routine basis.

Two-dimensional electrophoresis in soybean is being evaluated as an aid in germplasm characterization. Further research is needed to

integrate the electrophoresis pattern with computer image analysis before progress can be made in using this technique commercially.

The polymerase chain reaction (PCR), in which specific short regions of a gene can be greatly amplified in vitro from as little as a single molecule of DNA, has been used in forensic identification of humans. This technique has potential application in plant research such as genotyping (allele-specific) of individual plants.

For the immediate future, traditional methods plus chemical methods will be used for distinguishing among soybean cultivars.

#### Acknowledgements:

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**Table 1. Identification of soybean cultivars using isoenzyme electrophoresis and morphological traits.**

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<u>Electrophoresis</u>	<u>Electrophoresis and morphology</u>
174 cultivars (6 seed/cultivar)	174 cultivars
11 enzymes (starch gel)	11 enzymes
112 homogenous cultivars	+ flower color + pubescence color + hilum color
62 isozymically heterogeneous cultivars (composed of 2 or more sublines)	95% of cultivars identified
77% of cultivars identified	

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From: B. J. Cardy and W. D. Beversdorf. 1984. Seed Science and Technology 12:943-954.

Table 2. Isozyme profiles of selected closely related soybean cultivars.

Cultivars	ISOZYME PATTERNS								
	Aco1	Aco2	Aco3	Aco4	Ap	Dia1	Enp	ldh1	ldh2
A.K. (Harrow)	A	<u>A</u>	A	C	B	2	B	B	A
A.K. (Kansas)	A	B	A	C	B	<u>1</u>	B	B	A
Illini	A	B	A	C	B	2	B	B	A
Harosoy	A	B	A	C	B	1	B	A	A
Harosoy 63	A	B	A	C	B	1	B	A	A
Clark	A	B	A	C	B	1	B	B	A
Clark 63	A	B	A	A	B	1	B	B	A
Corsoy	A	B	A	C	B	1	A	B	A
Corsoy 79	A	B	A	C	B	<u>2</u>	<u>B</u>	B	A

J. D. Griffin and R. G. Palmer (unpublished data).

**Table 3. Current and expected future use of molecular markers in soybean. Response from a telephone survey to 10 companies engaged in soybean breeding.**

	Present			Future			
	<u>Response</u>	<u>Peroxidase</u>	<u>Isozymes</u>	<u>RFLP</u>	<u>Peroxidase</u>	<u>Isozymes</u>	<u>RFLP</u>
Yes		6	3*	1	8	10	10
No		4	7	9	2	0	0

\*Two companies only test for 'A' and 'B' protein.

**Table 4. Percentage contribution of ancestral strains to the 'collective' genome of 136 soybean cultivars of hybrid origin.**

<u>Ancestor</u>	<u>1939-81 Releases</u> -----	%	<u>1971-81 Releases</u> -----
Mandarin	17		14
Manchu	16		13
Mandarin (Ottawa)	13		17
Richland	11		11
A.K. (Harrow)	6		9
Mukden	5		4
Illini	5		5
Ogden	4		6
Dunfield	3		3
Strain no. 171	2		4
CNS	2		4
Manitoba Brown	2		1
Others	14		9
-----			
Total %	100		100

From: J. E. Specht and J. E. Williams. 1983. Genetic contributions to yield gains of major crop plants. pp. 49-74. American Society of Agronomy, Madison, Wisconsin.

**Table 5. Mean peak area percentages\* and standard errors.**

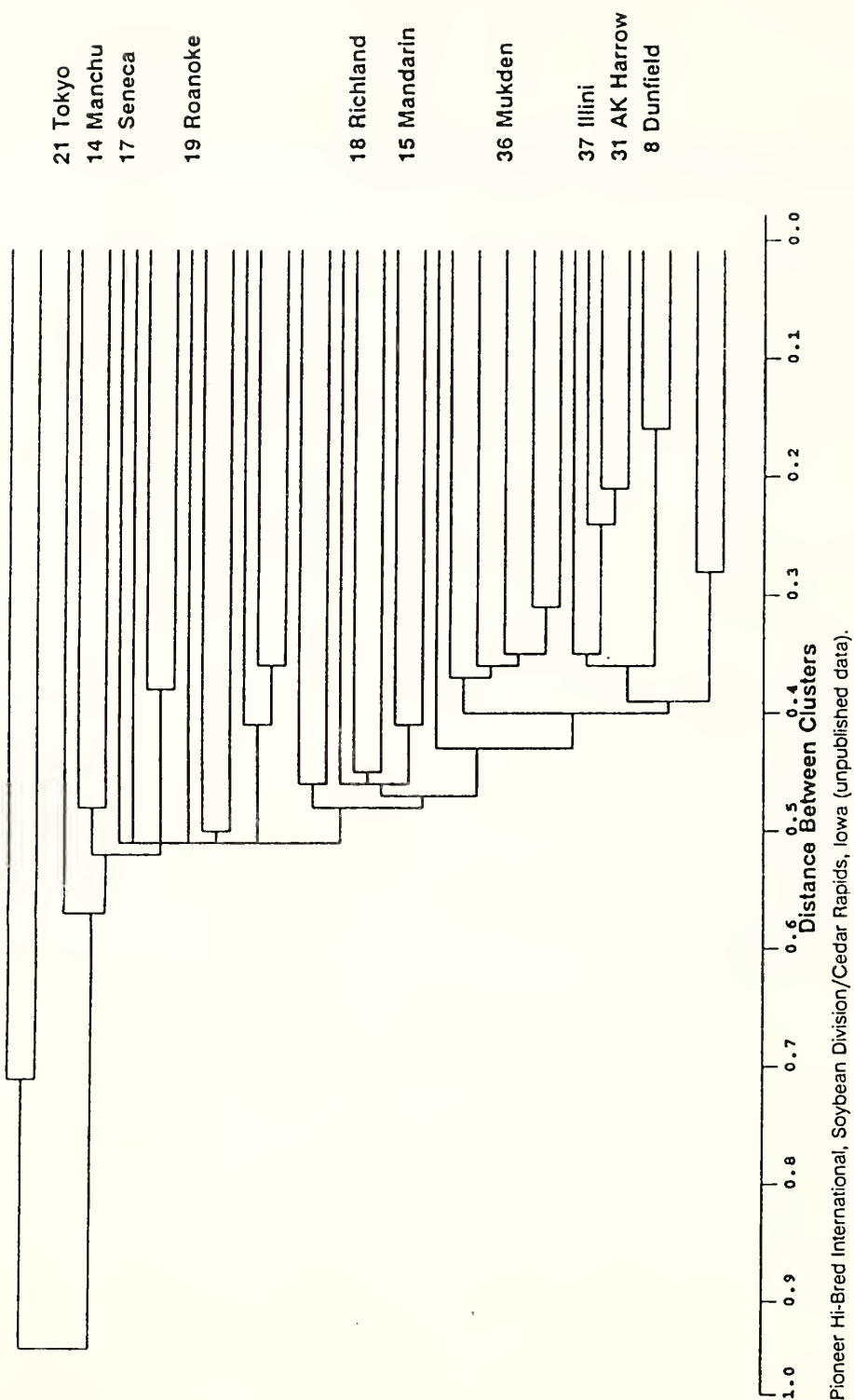
<b>Peak</b>	<b>Sprite</b>	<b>Hobbit</b>	<b>Williams</b>	<b>Williams 79</b>
<b>A</b>	<b>23.19±0.14</b>	<b>19.60±1.25</b>	<b>19.45±0.80</b>	<b>19.45±2.60</b>
<b>C</b>	<b>13.30±1.15</b>	<b>12.55±0.67</b>	<b>17.36±1.94</b>	<b>17.29±0.31</b>
<b>D</b>	<b>11.19±0.96</b>	<b>9.31±0.96</b>	<b>10.38±0.86</b>	<b>9.53±1.20</b>
<b>K</b>	<b>2.79±0.29</b>	<b>4.13±0.53</b>	<b>3.33±0.24</b>	<b>3.52±0.22</b>
<b>L</b>	<b>5.55±0.72</b>	<b>5.34±0.80</b>	<b>5.28±0.82</b>	<b>4.96±0.84</b>
<b>M</b>	<b>6.70±0.49</b>	<b>8.19±1.98</b>	<b>6.63±1.07</b>	<b>7.21±1.24</b>
<b>P</b>	<b>7.96±0.75</b>	<b>9.84±1.52</b>	<b>7.40±0.59</b>	<b>8.46±0.30</b>
<b>J</b>		<b>1.00±0.05</b>		

**\*Peak area percentages computes the area of selected peaks compared to the total area of all peaks.**

**From: R. E. Buehler, M. B. McDonald, Jr., T. T. Van Toai, and S. K. St. Martin. 1989. Crop Science 29:32-37.**



Figure 1. Cluster for Pioneer soybean study - RFLP data.



Electrophoretic Determination of Distinctness  
in Soybean Varieties  
(Summary)

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Before electrophoresis can be used to determine distinctness in a crop, testing procedures should be standardized. The standardization of testing procedures will insure that information from different sources is comparable. A number of different options for a standard electrophoretic procedure for soybeans were discussed. These options included: the types of gels and equipment available, testing seeds or seedlings, staining for proteins or isozymes, testing individual seeds and seedlings or bulk samples, the qualitative or quantitative interpretation of data, the necessity of a genetic analysis, and procedures to describe protein and enzyme bands and determine varietal distinctness.

Suggestions for a testing procedure appropriate for determining the distinctness of soybean cultivars were made.

Report of the American Seed Trade Subcommittee  
on Minimum Distance Determination  
in Soya Bean

Dr. John A. Schillinger

Executive Director of Agronomic Research  
Asgrow Seed Co.

(Full report not published here.)

Influence of Environment on Selected  
Plant and Seed Traits in Soybean

C.E. Caviness, Agronomy Department,  
University of Arkansas

(Presented at "Workshop on the Examination of Varieties of Soya Bean" on  
September 27, 1989 at New Carrollton, Maryland.)

Several characteristics such as plant height, growth type, leaf shape, maturity date, seed size, and chemical tests of seeds are used to identify offtype plants and seed in soybean programs. Major emphasis has been placed on flower and pubescence color of plants and hilum color of seed in most seed certification programs. Identification of flower and pubescence colors of most soybean cultivars is relatively easy but some variation generally occurs. Probably the most variable trait of those commonly used in certification programs is hilum color of seed of some genotypes.

Most cultivars have either purple ( $\underline{W}_1$ ) or white ( $\underline{w}_1$ ) flowers. There is a pleiotropic effect of the  $\underline{W}_1$  allele, in that purple pigmentation is evident on the hypocotyl of seedlings. The hypocotyl of seedlings with the  $\underline{w}_1$  allele is green. Hartwig and Hinson (4) reported that two additional genes designated  $\underline{W}_3$  and  $\underline{W}_4$  affect intensity of purple pigmentation in a few cultivars. Also, Buzzell et al. (2) reported that magenta flower color is controlled by the mutant allele  $\underline{wn}$  in the presence of  $\underline{W}_1$ . Flower color is a relatively stable trait that is not affected greatly by environment, although there are a number of genetic variations of flower color in soybean.

Pubescence color of most soybean cultivars is controlled by a single gene pair, with tawny or brown ( $\underline{I}$ ) dominant to gray ( $\underline{i}$ ). The

trichomes on young plants of the tawny pubescent genotypes are colorless, but after several weeks of growth, most of the trichomes on stems, pods, and leaves have brown pigment. Among gray pubescent cultivars, most trichomes do not have brown pigment which gives a distinct gray phenotype.

Bernard (1) in 1975 described another major gene pair that affects pubescence color. Id controls dark-tawny and td controls light tawny in the presence of I. Palmer and Payne (6) reported that dark-tawny (I-Id-) pubescent genotypes and light-tawny (I- td td) pubescent genotypes, when grown in continuous light, had bronze pigmentation on the hypocotyl shortly after emergence. Gray (t t Id - or t t td td) pubescent genotypes had no detectable bronze pigmentation.

Pubescence color is a relatively stable trait but appears to be affected more by environment than flower color. But, in general, certification officials seldom have difficulty in identification of most pubescent offtypes.

A number of researchers have conducted genetic studies on loci that affect pigmentation of soybean seed (5, 8). There is a pleiotropic effect of the I - t gene for pubescence color and seed color (Table 1). Seeds from tawny pubescent plants have black or brown pigment in the seed whereas gray pubescent genotypes have imperfect black or buff pigment. The Id gene does not affect pigmentation.

Table 1. Inheritance of hilum color and seed-coat pigmentation in soybean<sup>1</sup>.

Genes	<u>Self color</u>	<u>Saddle color</u>	<u>Hilum color</u>	<u>Hilum color</u>
	<u>i</u>	<u>i<sup>k</sup></u>	<u>i<sup>i</sup></u>	<u>I</u>
TR		black		gray
TrO		brown		yellow
Tro		red brown		yellow
tRW <sub>1</sub>		imperfect black		gray
tRw <sub>1</sub>		buff		yellow
tr		buff		yellow

<sup>1</sup>Data reported by R.G. Palmer and T.C. Kilen in review of "Qualitative Genetics and Cytogenetics in Soybean: Improvement, Production, and Uses. Agronomy No. 16, 1987. p. 170.

A multiple allelic series at the I locus conditions seed coat and hilum color in soybean (Table 1). Self-colored seed have the i allele. The k allele restricts pigmentation to a saddle pattern and i<sup>i</sup> restricts color to the hilum. The I allele inhibits the formation of certain of the pigments. Most commercial cultivars have yellow seed coats and the gene i<sup>i</sup> that restricts color to the hilum; however, a few cultivars have an inhibitor gene I that produces colorless hila with brown or buff genotypes and gray hila with black or imperfect black types.

Certain hila colors have been found to vary more than others and this has caused problems in seed certification programs. Williams (8) described imperfect black coats and noted that the color ranged from almost totally black to almost totally buff. Plants must have purple flowers, gray pubescence, and the R gene to produce seed with imperfect black hila.



Taylor and Caviness (7) studied hila variation in Pickett 71, which normally produces seed with imperfect black hila. They clearly showed that visual selection of buff offtypes from the Pickett 71 cultivar was not an accurate method of identifying hilum genotypes.

Seed of the imperfect black genotypes frequently have hila ranging from light buff to imperfect black. Research by Taylor and Caviness (7) showed imperfect black genotypes produce a buff pigment and, in normal development, an anthocyanin pigment masks the buff in the center of the hilum. But under conditions where seeds do not undergo normal development, failure of anthocyanin synthesis cause seeds to have buff hila. Often, a single plant from an imperfect black genotype may have seeds with hila colors ranging from dark imperfect black to very light imperfect black or even buff.

Taylor and Caviness (7) suggested that if the shape and size of the apparent offtype hila are similar to imperfect black types, the best method for quickly determining offtypes is to determine the flower color of the apparent offtype. Hypocotyl color can be determined by planting the putative offtype seeds and growing the seedlings under sufficiently intense light for anthocyanin development in the hypocotyl. If the seedlings have green hypocotyls (white flowers), the seeds are offtypes because imperfect black genotypes must have purple flowers. But, if the seedlings have purple hypocotyls (purple flower), the seeds probably are not offtypes but one cannot be absolutely sure.

#### Leaflet Shape and Number

Leaflet shape often is used to describe soybean cultivars. The ovate leaflet trait has been shown to be dominant to narrow leaflet with

a single gene involved. A number of other genetic types have been described and are found in cultivars. Leaflet shape is a relatively stable genetic trait, but there is considerable environmental variability within homozygous genotypes. This trait is much more variable than either flower or pubescence color.

Genotypes that have leaves with more than three leaflets are present in the germplasm collection and this trait is being evaluated in some breeding programs. Cultivars in the future may be released that have the multiple leaflet trait. Fehr (3) reported on inheritance of multiple leaflets and found that the dominant gene  $\underline{L}f_1$  was responsible for the five leaflet trait and a recessive gene  $\underline{l}f_2$  conditioned inheritance of a genotype with seven leaflets.

There is considerable variation in leaflet number of the multiple leaflet types with different leaflet numbers being present on individual plants. This is very different from the trifoliate genotypes where all of the leaves have three leaflets.

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## Breeding Methodology and its Role in Cultivar Protection

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### Abstract

Soybean cultivars in the U.S. are developed from three kinds of crosses, 1) two- and three-parent crosses, 2) multi-parent crosses, and 3) backcrosses. Two- and three-parent crosses have been the most productive source of new cultivars, followed by backcrosses and multi-parent crosses. Soybean breeders put major emphasis on agronomic traits, pest resistance and then on chemical composition of seed. Unique traits for cultivar identification are determined only after a superior breeding line has been developed. Public and private soybean breeders have agreed on a policy of ethical use of cultivars and advanced breeding lines in improvement programs to avoid the release of essentially-derived cultivars from competing research programs.

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Soybean breeders in the U.S. have, as their primary objective, the development of high-yielding, lodging-resistant soybean cultivars. Secondary objectives in their breeding programs have been the incorporation into new cultivars, resistance to major diseases, primarily *Phytophthora* rot, caused by *Phytophthora megasperma* Drechs. f. sp. glycinea Kuan and Erwin, and to the soybean cyst nematode, Heterodera glycines Ichinohe. Soybean breeders put virtually no emphasis on breeding for distinctive traits that will facilitate obtaining protection under the Plant Variety Protection Act. Attention

is focused on determining unique traits for plant variety protection of a cultivar only after a superior breeding line has been identified.

Three kinds of crosses are used by soybean breeders to develop new, favorable gene combinations for cultivar development. These include 1) two- or three-parent crosses, 2) multi-parent crosses, as used in recurrent selection, and 3) backcrossing. Each of these methods has somewhat different final objectives in terms of new cultivar characteristics and each affects the potential for obtaining Plant Variety Protection of germplasm resulting from the use of the method.

#### Two- and Three-Parent Crosses

This is the most common breeding method used for new cultivar development and has resulted in the largest proportion of new soybean cultivars released in the U.S. With more than 10,000 accessions in the U.S. soybean germplasm collection available for use as parents, one would expect ample genetic variability for both agronomic traits and for unique identifying traits from two- or three-parent crosses using these accessions. However, the germplasm base of U.S. soybean cultivars is, for several reasons, very narrow and this limits the genetic variability for both agronomic traits and for traits that can be used in cultivar identification.

A 1972 National Academy of Sciences report pointed out that 62 soybean cultivars grown at that time traced their ancestry to 29 plant introductions (6). Most of the ancestry of the commonly grown cultivars could be related to only 11 accessions. Since that report, cultivars and elite breeding lines derived from those accessions continued to be



used as parents for subsequent cultivar development, limiting the genetic variability available for soybean improvement and for cultivar identification.

In a single year, probably several hundred different two- and three-parent crosses are collectively made by soybean breeders. However, only a very small proportion of these crosses produce elite breeding lines or cultivars and this further limits genetic variability in soybean. Frequently, two or more cultivars are selected from progenies of a single two- or three-parent cross while all progenies of most other crosses are discarded. An excellent example of a productive two-parent cross that resulted in the release of five cultivars is the cross Lincoln x (Lincoln x Richland). Characteristics of cultivars selected from this cross are listed below.

Cultivar	Ref.	Flower color	Pubesc. color	Pod color	Seed luster	Hilum color	Maturity group
Chippewa	(9)	purple	tawny	brown	shiny	black	I
Renville	(13)	white	gray	brown	shiny	buff	I
Ford	(11)	white	tawny	brown	shiny	black	III
Shelby	(12)	purple	tawny	brown	dull	black	III
Clark	(10)	purple	tawny	brown	dull	black	IV

The cultivars Chippewa, Renville, and Ford could be identified from the commonly used morphological traits listed above. However, the agronomic trait, maturity, is needed to distinguish between the cultivars Shelby and Clark.

Some crosses have produced cultivars that are indistinguishable based on commonly used morphological traits. An example of this is the cross C1266R x C1253 that produced two cultivars, Wells and Bonus.



Cultivar	Ref.	Flower color	Pubesc. color	Pod color	Seed luster	Hilum color	Phytoph. resist.
Wells	(15)	purple	gray	brown	shiny	imp blk	Rps1
Bonus	(14)	purple	gray	brown	shiny	imp blk	Rps1

Wells and Bonus are identical in the morphological characteristics listed above and in reaction to the pathogen causing Phytophthora rot. Wells is an early maturity group II cultivar and Bonus an early group IV cultivar. The agronomic traits maturity, plant height, and lodging resistance were used to distinguish between these two cultivars.

#### Multi-Parent Crosses

Multi-parent crosses are commonly used to develop base populations for recurrent selection. Recurrent selection involves 1) intermating selected parents, 2) evaluating progenies from the intermatings, and 3) selection of desirable recombinants for another cycle of intermating. The objective of recurrent selection is to accumulate genes in the population that favorably affect the expression of a quantitative trait such as seed yield. With successive cycles of recurrent selection for an agronomic trait, the population may become more homogeneous for morphological traits that are used for cultivar identification.

Cultivars may be selected from any recurrent selection cycle that produces recombinants with superior attributes. Some additional inbreeding and selection may be required in these superior recombinants to produce a cultivar that is homogeneous for both identifying and agronomic traits. Recurrent selection in soybean has produced germplasm with unique attributes but has not been a productive breeding method of

developing superior cultivars. An example of a soybean cultivar developed directly from a recurrent selection population is Elgin, from the recurrent population A6 (7).

### Backcrossing

Backcrossing is a breeding method in which a specific trait from a donor parent is transferred to a desirable cultivar by repeated crossing to the cultivar (recurrent parent), and selecting for the specific trait after each cross. The objective is to recover the phenotype of the recurrent parent, plus the specific trait from the donor parent. Backcrossing has been used extensively in soybean breeding to add single genes for disease resistance to productive cultivars. Traditionally, the year of release of the backcross-derived cultivar is added to the name of the original cultivar, e.g. Amsoy 71, Century 84, or Pella 86.

Backcrossing is a breeding method in which success in developing an improved cultivar is assured, assuming a good cultivar is selected as the recurrent parent. A unique characteristic of the backcross-derived cultivar is easily demonstrated for plant variety protection, since the specific trait added from the donor parent serves as the distinguishing trait. An example of a series of backcross-derived cultivars is Williams (2), Williams 79 (Rps1-c) (4), Williams 82 (Rps1-k) (5), and Winchester (Rps1-b, Rps3) (1). In this series, the gene for *Phytophthora* rot resistance transferred into each cultivar in the series is shown in parentheses. The response of each cultivar isolate to a

series of soybean strains used as race differentials is evidence of the unique trait of each cultivar in the series.

Occasionally, different breeders may backcross the same trait into the same recurrent parent. When this has occurred, plant variety protection can be obtained for only one of the backcross-derived cultivars. The cultivar Vickery (8) was developed using four backcrosses to transfer the *Rps1-c* gene for *Phytophthora* rot resistance into the cultivar Corsoy. Vickery was protected under the plant variety protection act. In a parallel program, the same gene was transferred into Corsoy using five backcrosses, and the resulting cultivar was released as Corsoy 79 (3). Both Vickery and Corsoy 79 are described as being indistinguishable from Corsoy except for the added resistance to *Phytophthora* rot. Since the same gene for resistance was added to the two cultivars, Corsoy 79 would not have qualified for plant variety protection, and this protection was not sought for Corsoy 79.

Backcrossing was used by the Soybean Research Foundation to transfer to soybean cultivars the gene ln for narrow leaflet. This resulted in a series of productive cultivars with a readily identifiable trait that could be used in marketing or in protecting the cultivars through plant variety protection. Examples of Soybean Research Foundation backcross-derived cultivars and their parentage include SRF 100 [Chippewa 64 (8) x D61-5141] and SRF 300 [Wayne (7) x D61-5141] (personal communication, S. Frank, Soybean Research Foundation).

The widely grown cultivar Asgrow A3127 was used as a recurrent parent in transferring the *Rps1-k* gene for resistance to multiple races of *P. megasperma* var. sojae from the breeding line L24. Four publicly

developed cultivars were released from the original cross and from the additional backcrosses (personal communication B. A. McBlain).

Cultivar	Parentage	Distinctive traits
A3127		
Resnik	A3127 (4) x L24	Rps1-k
Flyer	A3127 (4) x L24	Rps1-k, maturity 4-5 days
GR8836	A3127 (4) x L24	Rps1-k, seed protein
GR8936	A3127 x L24	Rps1-k, white flowers

Resistance to *Phytophthora* rot distinguished the four cultivars from A3127. The white flower trait of GR8936 distinguished this cultivar from both A3127 and from the three backcross derivatives of A3127. The three backcross derivatives, Resnik, Flyer, and GR8836, could be distinguished by modest differences in the quantitative traits, maturity and seed protein content.

Publicly supported soybean breeders have evaluated their advanced breeding lines in multi-state and multi-province cooperative tests known as the Uniform Tests. One group of tests is collectively known as The Uniform Soybean Tests Northern States and the other as The Uniform Soybean Tests Southern Region. Participants in these tests have been able to use the advanced breeding lines in the tests as parents in their breeding programs. This has assured the rapid and widespread use of the best breeding lines that have been available.

With the advent of privately supported breeding programs, both public and private soybean breeders freely used released cultivars from both kinds of programs as parental material in their breeding programs. In a few instances, reselection within released cultivars or, more

commonly, backcrossing specific traits into released cultivars, were methods used by breeders to develop and release slightly modified versions of competitors cultivars. Some of these changes were cosmetic, some slightly affected agronomic performance, while others, such as disease resistance, added economic value to the modified cultivar.

Reselection within cultivars, or backcrossing to cultivars developed by different breeders, has stimulated the development of policies to limit these practices. Public and private soybean breeders, in joint meetings, have agreed upon a policy for acceptable use of germplasm from different breeding programs. Cultivars or breeding lines may be used by soybean breeders only in two-parent crosses or as parents in the development of recurrent selection populations. Reselection within cultivars or breeding lines is not permitted, nor is the use of cultivars or breeding lines as recurrent parents in backcrossing, or in tissue culture applications, without the consent of the developer of the cultivar or breeding line. This policy will prevent the release of a cultivar that is essentially derived from an existing cultivar or breeding line without the permission of the breeder of the original cultivar or line.

With few exceptions, both publicly and privately supported soybean breeders agree that free and open exchange of soybean germplasm is essential for maximum genetic gains in soybean improvement. It is hoped that the policy permitting the use of cultivars or breeding lines in two-parent crosses will continue to foster the free exchange of soybean germplasm. Continued progress in maximizing genetic gains in soybean



improvement will be essential to maintain the position of soybean among competing sources of vegetable oils.

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## Selection criteria in U.S. soybean breeding programs

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The objective of this paper is to summarize the major selection criteria used in the development of U.S. soybean cultivars. The primary selection goal is a cultivar for use in a monocropping system. Substantial soybean acreage is also planted as the second crop in a field during a year. When soybeans follow a mature crop it is called doublecropping or when planted into an immature crop that is usually a small grain such as winter wheat it is called intercropping. Considerable discussion has suggested the merit of developing cultivars for doublecropping but only 'Duocrop' has been released specifically for double-crop plantings.

Cultivar development is the goal of both public and private soybean research programs. The public programs consist of USDA and university personnel. Cultivar development does not receive as much emphasis in some public programs as is placed on basic germplasm evaluation and enhancement. Private companies have substantially increased their cultivar development efforts since the Plant Variety Protection Act in

1970. Currently almost 60% of the U.S. soybean acreage is planted in privately developed cultivars. Growers quickly accept new cultivars, but 40 to 60% save their own grain for seed use the following year. This has contributed to a proliferation of new cultivars and reduced the length of time many cultivars are marketed.

### Seed Yield

The primary selection criterion in soybean breeding programs is seed yield. Considerable evaluation is conducted to identify experimental lines that have broad adaptation. Excellent yields in multi-locations and multi-years are required before an experimental line is considered for release as a new cultivar. In addition to seed yield per se, an experimental line needs to have an erect growth habit to permit maximum pod formation and to facilitate mechanical harvest. Pods must also resist shattering following plant maturity to allow growers sufficient time to harvest the crop.

### Maturity

Cultivars are adapted to narrow bands of latitude due to their photoperiodic response. Plant breeders in the U.S. have assigned cultivars to thirteen maturity groups with the designations of 000, 00, 0, and I through X. Cultivars are assigned to maturity groups based on their relative maturity to standard cultivars in each group. Some breeders use a relative maturity index to assign a maturity designation to each cultivar, but either system utilizes standard cultivars in deciding maturity classifications.

### Stem Growth

Most cultivars in the U.S. have either an indeterminate or a determinate stem growth type. Indeterminate plants continue to increase in height after flowering is initiated. Determinate plants essentially cease stem growth when flowering commences. A semi-determinate growth habit that is phenotypically between the determinate and indeterminate types is present in a few cultivars (Bernard, 1972).

Stem growth had traditionally been associated with maturity group prior to the 1970's. Cultivars of maturity groups IV and earlier are generally indeterminate in growth habit while those cultivars of maturity groups V and later are generally determinate in growth habit. This association is not exact since breeders have developed determinate cultivars in the early maturity groups and indeterminate cultivars in the late maturity groups. Selection programs to incorporate the genes for a brachytic (Kilen, 1977; Boerma and Jones, 1978) and a fasciated (Albertsen et al., 1983) stem growth type may result in cultivars with these traits in the future.

### Pest Resistance

Disease resistance is an important selection criterion in U.S. breeding programs, although the primary diseases differ by region. Phytophthora rot caused by Phytophthora megasperma, f. sp. glycinea (Pmg) is one disease that receives considerable emphasis in many cultivar development programs. Eleven different alleles have been identified that impart resistance to one or more races of the pathogen

(Athow, 1987). The use of single genes for resistance has resulted in a continuous increase in new races of the pathogen. Breeders have begun to combine multiple genes that impart resistance to many of the 26 Pmg races into a cultivar as a new strategy to control this disease.

Cultivars have also been identified that have tolerance to the pathogen. Although it is generally thought that tolerance is due to a large number of genes and is not race-specific in its expression (Walker and Schmitthenner, 1984), there is some recent information to suggest that the plant tolerant reaction may exhibit race-specificity in its expression (Thomison et al., 1988).

Resistance to several nematode species is also an important selection goal in many U.S. breeding programs (Riggs and Schmitt, 1987). At least five races of cyst nematode (Heterodera glycines) cause yield reductions in several U.S. soybean production areas. Four genes that impart race-specific resistance have been used in the development of productive resistant cultivars. Resistance to several root-knot nematode species (Meloidogyne spp.) has been incorporated in many cultivars for the southeastern U.S.

Cultivars such as Lamar with resistance to several foliar-feeding insects have only recently been released for production in the South. These areas receive damage from several insect pests (Turnipseed and Kogan, 1987) that exceeds economic threshold levels that require chemical control in most years. Resistant cultivars offer the growers the advantage of eliminating the costly application of insecticides, but have been difficult to develop.

### Environmental Tolerance

Cultivars have been identified with tolerance to special environmental conditions such as the high calcareous soils that cause iron deficiency chlorosis in areas of the Midwest and to several herbicides. Cultivars differ in their expression of iron-deficiency chlorosis. Screening techniques have been developed (Coulombe et al., 1984) and selection for improved resistance to iron chlorosis has been initiated (Fehr, 1982). Genes for tolerance to the herbicides bentazon (Bernard and Wax, 1975) and metribuzin (Edwards et al., 1976) have been identified in the germplasm collection. Herbicide tolerance will most likely continue to receive emphasis in breeding programs as novel gene transfer techniques permit the addition of new sources of tolerance to herbicides that are phytotoxic to soybeans.

### Seed Chemical Composition

The seed chemical composition of released cultivars has not been of concern to breeders as long as new cultivars did not deviate significantly from those currently grown. Greater emphasis on protein and oil composition of new cultivars will be noted in the future. The soybean industry has suggested that U.S. cultivars should have higher protein content to be more competitive with foreign produced soybeans in the export market. The Federal Grain Inspection Service will offer oil and protein testing as official criteria effective September 4, 1989, for growers to see chemical composition of current cultivars. The American Soybean Association has suggested that breeders develop cultivars that have protein contents greater than 41% and oil content of



less than 21% with the total of both constituents being at least 62% on a dry weight basis. These selection goals for chemical composition could have a dramatic impact on U.S. breeding programs.

Negative correlations between protein and oil composition and between protein and seed yield (Burton, 1987) will increase the difficulty in meeting these goals for new cultivars. Growers will not accept new cultivars with high protein levels if yield is below currently grown cultivars unless there is a premium paid for high protein grain.

#### Other Characteristics

Several other cultivar characteristics are used in descriptions, but generally receive little attention in most cultivar development programs. These characteristics are seed size and appearance (luster and visual quality); color of plant pubescence, pod wall, flower, foliage, seed and seed hilum; mature plant height; and shape of the plant and leaves.

Soybean breeders have contributed to the plant expansion of U.S. soybean production by developing productive cultivars adapted to a range of environments and having resistance to the major pests limiting yields. Breeders will be able to utilize novel gene transfer techniques and new germplasm resources (Kenworthy, 1989) to assist them in developing cultivars to meet the production challenges of the next decade.

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## Morphological Traits Used to Differentiate Soybean Varieties

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For the purposes of this presentation I will be defining morphology to include not only the differences in plant structures of the soybean but also the pigmentation of those structures. Variation in both of these categories is very useful to differentiate soybean cultivars. Because the maximum range of variability for most characteristics, especially qualitative traits, will be found within germplasm collections, I will base this presentation on our experience in evaluating the USDA Soybean Germplasm Collection (Nelson et al., 1987, Nelson et al., 1988, and Juvik et al., 1989). Not all of the variation observed within the collection is going to be available in commercial cultivars but as the genetic base of commercial cultivars is expanded, the diversity within the common descriptors will also increase. Knowledge of the variation that exists within the species will provide a

good basis for discussion on the potential and the problems associated with morphological descriptors both now and in the future. Additional information on genetic control of morphological traits as well as other qualitative traits in soybean is given by Palmer and Kilen (1987).

Before I begin descriptions of specific traits, I will comment generally about the uses and limitations of morphological traits. Most of the morphological descriptors that are currently used for soybean have been studied genetically and have been shown to be controlled by a very limited number of loci. This information is extremely useful, but it may also tend to lull us into over-simplification in describing phenotypes. If we know that a single gene pair has a major effect on a given trait, that should not exclude from consideration other loci or alleles that may have significant influences on the phenotype. Separating these effects from those of the environment also adds to the complexity. Nature is notoriously difficult to categorize. If we could examine the range of genetic variability within the species for the qualitative descriptors that we commonly use in soybean, I would not be surprised to find a continuum for many traits. Pod walls do not occur in only three colors, and the boundaries between leaflet shape types are not unambiguous. I do not mean to imply that morphological traits are not useful. They have been and will most certainly continue to be. I do mean that our expectations for these descriptors should be tempered. Firstly, not all observed variation will be useful for cultivar description. Variation that is not consistently expressed, that is difficult to define, or that can not be easily and accurately classified will be of limited value. Secondly, classification schemes may have

ambiguous boundaries between classes. For those traits there would be no doubt about the archetypical specimen for each category but uncertainty would exist as to the proper classification of some intermediate types. Finally, if the identity of a cultivar is in dispute, descriptors that are to be useful will need to be defined in objective terms. Tall and short or small and large will not be adequate. All of these factors need to be considered as we select the morphological factors to describe soybean cultivars and as we define the classes for each factor.

#### Flower Color

Within the U.S. soybean cultivars three flower color types are currently used: white, purple, and purple throat. The latter is mostly white with purple coloring at the base of the standard petal. The genetic control of these colors have been described with alleles at three loci (Hartwig and Hinson, 1962). White flowers (w1) and purple flowers (w1) are controlled by alleles at a single locus. Two additional loci (w3, w4) are involved in the purple throat phenotype. A fourth locus (w2) that affects the color of purple was named in 1933 (Matsuura) but that work has never been confirmed and the genetic sources used in that work are not now available. There are several distinct shades of purple that are observed in the germplasm. Some of these could be explained using combinations of known genes but the genetic control of these types has not been studied. Both a dark and a light shade of purple are routinely classified in the germplasm collection. These extremes are very distinct but relatively rare.



Buzzell et al. (1977) reported on the inheritance of a magenta flower color but this is a very rare flower color.

#### Pubescence Color

Tawny (brown) and gray pubescence are controlled by a single gene pair and most cultivars fit one of those categories. The genetic control of a third type, light tawny, is known (Bernard, 1975a) and within that category variation can be observed. Light tawny and near gray are used in the germplasm collection to describe intermediate levels of pigmentation between tawny and gray. Variation about cultivars may not warrant four categories for this trait.

#### Pubescence Form

There are five categories for this trait within the germplasm collection: appressed, semi-appressed, curly, irregular, and erect. Nearly all cultivars grown in the northern U.S. have erect pubescence. Curly (Bernard and Singh, 1969) and irregular types have varying levels of deleterious effects primarily because of susceptibility to the potato leaf hopper (Empoasca fabae). Curly pubescence is twisted and appressed against the plant surface. Irregular pubescence is slightly twisted and appressed but the genetic analysis of that trait has not been published. Appressed and semi-appressed types (Bernard, 1975c) may sometimes also show damage from the potato leaf hopper. In addition, sharp and blunt pubescence tips are controlled by a single gene pair (Ting, 1946). This characteristic must be observed under magnification and has not been widely used.

### Pubescence Density

Five levels of pubescence density have been documented through genetic analysis (Bernard and Singh, 1969) and are used in the evaluation of soybean germplasm. In order of decreasing density they are: dense, normal, semi-sparse, sparse, and glabrous. A single gene pair controlling the sparse level of pubescence is known but there is variation in the amount of pubescence for types classified as sparse. Nearly all U.S. commercial cultivars have normal levels of pubescence but some recent Canadian cultivars have semi-sparse levels of pubescence. The semi-sparse level may have only a small reduction of pubescence density on the leaf and stem but has a large reduction on the pulvinis (Bernard, 1975b). Certain cultivars and introductions have been classified as semi-dense but the genetic differences between dense and semi-dense have not been published. Sparse and glabrous types are also adversely affected by the potato leaf hopper and thus will not be in commercial cultivars for areas in which that insect is present.

### Leaf Characteristics

Published studies provide the genetic explanation for three types of leaflet shape (Domingo, 1945): lanceolate, ovate, and oval. Oval leaflets occur very rarely in the species. Within the lanceolate and ovate types, there is a wide range of leaf dimensions. Mutations that can change leaflet number are also known (Fehr, 1972). Wavy leaflet margins (Rode and Bernard, 1975) and delayed leaf abscission (Probst, 1950) have also been studied genetically and are routinely classified in

the germplasm. Although variation exists for both leaflet size and color, the germplasm has not been routinely classified for either of these traits because of the difficulty in objectively defining these categories.

#### Stem Termination

Stem termination is controlled by two pairs of major genes (Bernard, 1972) and most probably by many minor modifying genes. In commercial cultivars it is treated as a qualitative trait being either determinate, semi-determinate, or indeterminate. Given the range of variability in the current cultivars for this characteristic those categories work well and one can probably deduce the stem termination genotype from the phenotype. In the germplasm collection, stem termination is scored as a quantitative trait and then only with great difficulty in some cases. Part of the problem occurs because there is no information about the actual anatomical differences among the stem termination types and thus no definitive descriptions of each type. Three standard characteristics are usually used to describe determinate plants: 1) main stem growth ceases at or shortly after flowering, 2) the stem terminates in a prominent raceme, 3) the mature stem terminus is thick and not tapering. Indeterminate plants may double in height after flowering, and the mature stem terminus is tapered and has no definitive end point. Semi-determinate types are intermediate between the determinate and indeterminate types. In the germplasm, there are types with viney, tapering stems that end with prominent racemes, as well as types that will double main stem height after flowering but will have a

prominent terminal raceme and thick main stem terminus. Current research on stem termination genetics and the development of experimental lines and cultivars with altered morphology may further define this issue.

#### Other Plant Characteristics

In the germplasm we have not attempted to classify plant canopy types but do characterize the canopy by measuring plant height. Variation in canopy shape does exist but working definitions of those differences have not been established for use within the germplasm. Relative maturity date in the form of maturity groups is used to differentiate among both commercial cultivars and germplasm accessions. Time of flowering is not necessarily related to time of maturity and can be measured as precisely as time of maturity (Nelson, 1988). Time of flowering is recorded on all germplasm evaluation but is rarely reported on commercial cultivars.

#### Pod Color

Genetic control has been established with two loci to give three pod colors: tan, brown, and black (Bernard, 1967). Because the black pod color usually causes discoloration of a yellow seed coat, commercial cultivars will probably remain only tan and brown. Both a dark and a light variation of brown are classified in germplasm evaluation.

### Seedcoat Luster

Because seedcoat luster is highly affected by the environment, it must be used carefully if it is to be an effective descriptor. In the germplasm collection there are four categories: bloom, dull, intermediate, and shiny. The latter three would apply to commercial cultivars. Without an intermediate category, this descriptor is not useable. In the classification of the germplasm the intermediate category is generally the largest. The extreme dull or shiny types tend to more consistently maintain that phenotype in different environments while the intermediate category represents a variable range between those two extremes.

### Seedcoat Color

More than a dozen different seedcoat colors are expressed in the germplasm collection but probably only two are needed to describe commercial cultivars: yellow and green. Shades of both of these colors are observed in the species. These differences have not been critically studied nor have they been used to differentiate among cultivars, although categories of green are used within the germplasm collection.

### Hilum Color

There is a great variation in hilum color that exists within the germplasm collection and commercial cultivars. Published genetic information can account for at least 8 colors (Palmer and Kilen, 1987): yellow, gray, black, brown, buff, imperfect black, reddish-brown, and green. Gray and sometimes imperfect black hilum can be quite variable



not only between seed lots but within seed lots. The classification of imperfect gray (produced by the same alleles as those that give imperfect black except that it occurs in plants with gray instead of tawny pubescence) is not always expressed but is used occasionally within the germplasm collection. Variation is observed for yellow hilum color but no sub-categories have been described. Reddish-brown is a quite rare type. For gray, black, brown, buff, and green hilum colors the modifier "light" is used in germplasm evaluation. The most common usage is with buff hilum color. In that case, light generally describes a range of hilum color intensity that may extend from lack of color (yellow) through a very intense buff although in some cases the hilum color may be uniformly less intense than normal. In the former case, no genetic explanation has been determined but selection for hilum color within these seed lots is unsuccessful in removing this phenotypic variability. Consistently less intense expression of gray, black, brown, and green are classified with the "light modifier although these types are quite rare in the collection. Only two shades, "normal" (no additional designation) and "light", have been used to describe hilum colors within the germplasm collection.

#### Seed Weight (Seed Size)

The weight of individual seeds is a quantitative trait with over a ten-fold difference within the species. Seed weight is affected by the environment in which the seed is grown and like most quantitative traits is a useful descriptor only in comparison with cultivars grown under similar conditions. With the recent increased interest in developing



both large- and small-seeded cultivars for human consumption the range of this trait within commercial cultivars is likely to increase.

#### Seed Shape

Large variation in seed shape occurs within the germplasm. Recent research by Nelson and Wang (1989) showed that all three dimensions of the soybean seed can vary independently. They established 15 classes of soybean seed shape based on the ratios of height to length and height to thickness. This research demonstrated that the seed could be visually classified and that seed shape was reasonably consistent across environments even when seed weight changed. The range of seed shape within current cultivars is much more limited but it may be a characteristic that could be further exploited.

#### Other Seed Traits

There are a variety of other distinctive seed traits that are observed in the species. These are classified in the germplasm when they appear but the normal phenotype is the predominant class. Imperfect abscission of the hilum from the pod (Owen, 1928) does occur in some cultivars. In these cases, part of the funiculus remains on the seed obscuring part of the hilum. The penetrance of this trait can be affected by the environment. Green cotyledon does occur in commercial cultivars and can be caused by either nuclear or cytoplasmic factors (Veatch and Woodworth, 1930). Other traits classified in the germplasm that affect seedcoat pigmentation or structure are not likely to occur in commercial cultivars.

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PVP and Intellectual Property Rights  
as They Involve Transgenic Soybean Varieties  
(Summary)

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Proprietary protection for plants in the United States is potentially available under three different statutes. The advantages and limitations of the plant patent statute, Plant Variety Protection Act and the utility patent statute will be briefly discussed. Proprietary protection under the utility patent statute affords, at this time, the only means to obtain generic protection for discoveries in plant sciences. Plants may be protected under the utility patent statute provided that the requirements of utility, novelty and non-obviousness are met. Furthermore, the statute requires the applicant to provide an enabling disclosure to teach those skilled in the art how to make and use the claimed invention. Deposit of biological material may supplement the written disclosure. Requirements for protection of plants under the utility patent statute will be discussed in detail.

## **PLANT PROTECTION**

**Plant Patent Act of 1930 (35 U.S.C. 161)**

**Plant Variety Protection Act of 1970  
(7 U.S.C. 2321)**

**Utility Patent Act of 1952 (35 U.S.C. 101)**

## **PLANT PATENT ACT OF 1930** **(35 U.S.C. 161)**

- Patentability - "New, Useful And Distinct"
  - New - no previous Existence
  - Distinct - Having Characteristics which clearly distinguish from existing Varieties
  - Enabling Description Waived  
(35 U.S.C. 162)
- Protects Asexually Produced New Varieties  
(Except Tubers)
- Right to Exclude Others for 17 Years From  
Asexually Reproducing the Plant or Selling  
or Using the Plant so Produced



## **PLANT VARIETY PROTECTION ACT OF 1970** **(7 U.S.. 2321)**

- **Protectability - "Novel, Distinct, Uniform and Stable"**
  - **"Novel"** - Not publicly known for more than one year
  - **"Distinct"** - Has one or more characteristics different from all publicly known varieties
  - **"Stable"** - Sexually reproducible with reasonable reliability while retaining its distinctive characteristics
  - **"Uniform"** - The degree of variation is commercially acceptable
- **Must Deposit Seeds**

## PLANT VARIETY PROTECTION ACT OF 1970 (7 U.S.C. 2321)

- Protects Sexually Produced New Varieties  
(Except Hybrids)
- Right to Exclude Others For 18 Years From  
Commercial Use of New Variety
- Exemptions
  - Research (novel varieties)
  - Farmer (save seed)

## **UTILITY PATENT ACT OF 1952** **(35 U.S.C. 101)**

- **Chakarbarty, 206 U.S.P.Q. 193 (1980)**
  - **Supreme Court held that genetically engineered bacterium was patentable subject matter**
  - **Congress intended "to include anything under the sun that is made by man"**
  
- **Ex Parte Hibberd et al, 227 U.S.P.Q. 443 (1985)**
  - **Patent Office refused patent on maize having high tryptophan**
  - **Board of Appeals ruled that plants were patentable subject matter**

## **UTILITY PATENT ACT OF 1952** **(35 U.S.C. 101)**

- **Patentability**
  - **New and Useful**
  - **Unobvious**
  - **Enabling written description**
  
- **Potential Exists to Protect Plants Generically Including Plant Parts and Genes**
  
- **Right to Exclude Others for 17 Years From Making, Using or Selling the Invention**

## PLANT PROTECTION

	<u>PPA</u>	<u>PVPA</u>	<u>UPA</u>
Novelty	✓	✓	✓
Unobviousness	✓		✓
Complete Written Description			✓
Generic Protection			✓
Protection For Genes			✓
Varietal Protection	✓	✓	✓

## WHAT IS A PATENT?

A patent is a grant, limited in time, of exclusive rights in an invention.

Congress was granted the power to create the patent laws under Article I, Section 8, of the Constitution, which provides:

"The Congress shall have the power....to promote the progress of.....useful arts, by securing for limited times to.....inventors the exclusive right to their respective.....discoveries."



## **PATENTS: AN EQUITABLE EXCHANGE OF BENEFITS**

**If an individual makes an invention, he has an option of either:**

**Gratuitously disclosing the invention to the public**

**Keeping the invention secret so no one else can use it**

**Teaching the public how to make and use the invention  
in return for a limited exclusive right in the invention**

### **Individual Receives:**

**Exclusive right in the invention for a period  
extending usually 17 years via a patent**

**Incentive to continue research**

### **Public Receives:**

**New information**

**Right to make and patent improvements on the  
invention**

**Right to freely practice the invention upon  
expiration of the patent**

## **WHAT RIGHT DOES A PATENT COVER ON THE PATENTEE OR ASSIGNEE OF THE PATENT**

Patents give owners the right to exclude others from practicing the claimed invention. Patents do not give the owner the right to practice the claimed invention. A person may own a valid patent but be restrained from practicing the invention by a valid blocking or dominating patent of another.

Why? - To protect the limited monopoly of the owner of the fundamental patent.

## **REQUIREMENTS OF A PATENTABLE INVENTION OR DISCOVERY**

Proper Subject Matter (35 USC 101)

New (35 USC 102)

Useful (35 USC 101)

Non-Obvious (35 USC 103)

## **PROPER SUBJECT MATTER**

### **Utility Patents (35 USC 101)**

- 1) **Process**  
Method of making something  
Method of using something
- 2) **Machine**
- 3) **Article of manufacture**
- 4) **Composition of matter**  
Diamond v. Chakrabarty - microbes  
Ex parte Hibberd - plants  
ex parte Allan - animals
- 5) **New and useful improvements thereof**

## **AN INVENTION IS NOT "NEW" IF:**

- Known or used in this country or patented or described in a printed publication anywhere before the date of invention of applicant
- Described in a printed publication anywhere, in public use or on sale more than one year before date of application
- The invention was described in a patent granted on an application by another filed in the U.S. before the invention by applicant

## **35 USC 103**

### **THE INVENTION MUST BE NON-OBVIOUS**

A patent may not be obtained....if....the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains, patentability shall not be negated by the manner in which the invention was made.



## **NON-OBVIOUS**

The determination as to whether a particular invention is obvious in view of the prior art is always a judgement call. Non-obviousness cannot be conclusively proven. However, recognized indicia of such include:

- 1) Unexpectedly good results
- 2) Long-felt need - failure of others
- 3) Commercial acquiescence - licensing
- 4) Professional approval

## **THE PATENT APPLICATION**

### **35 USC 111**

**Application for patent shall be made, or authorized to be made, by the inventor, except as otherwise provided in this title, in writing to the Commissioner. The application shall include:**

- 1) A specification as prescribed by 35 USC 112**
- 2) A drawing where necessary for the understanding of the subject matter sought to be patented**
- 3) An oath by the applicant as prescribed by 35 USC 113**
- 4) The fee required by law**

**THE PATENT STATUTE (35 USC 112)**  
**REQUIRES THAT THE**  
**SPECIFICATION CONTAIN:**

A written description of the claimed invention, and

A description of how to make and use the claimed invention in such full, clear, and concise terms to enable those skilled in the art to make and use the invention.

The description shall set forth the best made contemplated by the inventor for carrying out the invention.

## **PLANT CLAIMS UNDER TITLE 35 USC**

- **Processes, genes, vectors, plant cells, plant tissue and whole plants**
  - **Genes and vectors are compounds per se**
  - **Plant cells, plant tissue and whole plants are compositions of matter not articles of manufacture**
- **Claims cannot embrace the prior art**

## **EXPERIMENTAL USE**

- The "experimental use" exception to utility patent infringement has evolved jurisprudentially
  - Narrower than the "research exception" of the PVPA
  - Permits one to practice the invention in a "legitimate" research endeavor to:
    - satisfy an intellectual curiosity
    - make "improvements" in the claimed invention
- "Improvement inventions" are statutory subject matter which may be separately protected by a utility patent
  - However, these inventions may be dominated

Case law does support the proposition that an inventor should be able to dominate future patentable inventions

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Those later inventions are based in some way on the inventor's teaching, and the later inventions were made possible by the first inventor's work

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However, the scope of the claims must be consistent with the scope of enablement

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Transcript of the  
Panel Discussion on

Minimum Distances Between Varieties

Barry Greengrass (UPOV): Ladies and gentlemen, to kick things off, it might be worthwhile if I just very briefly remind you about the program, what we've heard, and the various elements. On the first day, I spoke briefly about the UPOV convention and distinctness, minimum distance questions and certain proposed revisions in the UPOV convention, particularly the question of dependence. We then heard a very clear description of the US system in the soybean computer database, followed by a description of the European system. The actual generation of the data in the two systems is very similar and the underlying questions, problems and so on are identical in Europe to those that you encounter here in the US. The sessions on future molecular tools from Dr. Reid Palmer followed by Dr. Payne's presentation on electrophoresis I think were very interesting and fitted in rather well with what we subsequently heard from Dr. Caviness about the morphological features that we normally use compared with the biochemical tools, and I think began to give us some ideas about how the two might be complementary to each other. Dr. Wilcox explained some of the breeding procedures and how these fitted in with variety protection. And we'd heard from Dr. Schillinger about the deliberations of the ASTA committee on minimum distances and their conclusions. Then on Thursday we had the presentation from Dr. Kenworthy on breeding objectives followed by what I think was a very interesting session with Dr. Nelson on morphological traits that we use. So where's this taken us? I think one of the main conclusions one has to reach is that within the existing system, as it stands, there are some problems. The problems arise from the fact that we ask this thing we call the minimum distance, or distinctness requirement to do two things. We're seeking really a technical determination that 'A' is clearly distinguishable from 'B'; that's one thing. But, with the existing legal provisions, we try to ask the amount of distinctness between two lines to also act as some sort of decision maker in relation to the ethical, value judgement question about whether, in fact, a variety 'B' is sufficiently original to be worth a grant of rights. And, insofar as we've had problems in the past with minimum distances, it's because we have asked the actual minimum distance between two varieties, the physical thing, to answer both those questions. We inevitably were drawn into discussions about the future and I think we felt that we couldn't sensibly carry forward discussions on minimum distances whilst ignoring the proposals for the amendment of the UPOV convention, particularly the introduction of dependence. Then we spent quite a lot of time talking about dependence and what this means. So that's a very brief introduction to the presentations and some of the discussion. I would invite you to comment on where you think we've arrived, either from the floor or from the panel.

Stanley Schlosser (Foley, Lardner, Schwartz, Jeffery, Schwaab): This, of course, is going to be a question that reeks of botanical naivete but I will ask it anyway. Is one of the problems of defining minimum

distances between varieties the fact that, at least in my mind and I suspect other minds, we don't know what a variety is? A variety is an artificial definition to begin with. And would this problem be easier if we had some clear definition, or at least some agreement, as to what a variety is? Then we would have a better chance of asking it to be easier to distinguish between varieties.

Barry Greengrass (UPOV): That particular issue, in relation to soybeans, I think in the discussions we've had, has not really been an issue. Because we're dealing with a more or less pure line in most cases. Most people present in the room understand what a soybean variety is. They may have different views about how uniform it should be, but that's not really been a big issue. It could be an issue of course in some other species, particularly cross-pollinating grasses for instance.

Stanley Schlosser (Foley, Lardner, Schwartz, Jeffrey, Schwaab): You said there is some disagreement as to how uniform it should be, but here we're talking about the differences between non-uniform varieties. It reminds me of the situation where, in the range of non-uniformity, one overlaps the other. Is that a problem?

Kenneth Evans (PVPO): In soybeans, that's no problem in our office as far as I know. Non-uniformity is probably limited to 5% or fewer of the plants and for all practical purposes when we examine that application we ignore that non-uniformity. So we examine the 95%; and if you got 5% of something else in there, we ignore that 5%. If somebody else comes in with another variety that has that same 95%, we would not issue it a certificate even though it had an entirely different 5% off-types or variants, whatever you want to call them. So for soybeans, that's not a problem. When you get into some other crops it can be more of a problem.

Andre Heitz (UPOV): Classically in UPOV, discussions on minimum distances are separated or, at least we try to separate it, into two issues. The first is: what characteristics are used to establish distinctness? The second question is: what is the magnitude of the difference which is required to establish distinctness for the purposes of granting rights and, if there is no inapplication, for the purposes of recognizing the new materials as something distinct from an existing, possibly protected, variety. If it is distinct, it is considered to be not infringing. If it is not distinct, in fact, it is considered under the prevalent doctrine within the western European countries as infringing the protected variety. I think we may perhaps address first the question of characteristics. It is a sort of agreement within UPOV that characteristics which are economically important and will have a bearing upon the commercial success of the variety should be used in all cases unless the cost/benefit ratio, and also the certainty of the decision which may be made on that characteristic, is not good enough. For example, to test winter hardiness was not accepted by the tribunal in the United Kingdom because that would have, in the opinion of that tribunal, required too great an effort from the Plant Variety Protection

Office in the United Kingdom. May I perhaps hear whether this is an opinion which is shared among this group in the United States of America?

Richard Payne (International Seed Testing Association): It seems to me that as time goes on there will be increasing pressure to minimize the minimum distance. I think that there'll be pressure from the seed industry itself for this so that they can patent an increasing number of varieties. From my own personal point of view, or shall I say from a scientific point of view, it would seem that a single gene difference should be sufficient, be that an enzyme band or an observable trait. But from a practical point of view, I think it should be an observable trait in the field or in the seed or something in the end product, such as a protein difference or a difference in the oil content of the seed.

Randall Nelson (USDA, ARS, University of Illinois): It seems to me that in trying to talk about minimum difference, if there is anything that we can sufficiently define, its not going to exceed the minimum difference. I think that that's a problem and I think that's why the ideas that have been brought up about 'essentially derived' and 'dependence' are going to be extremely important and, until and unless we include the pedigrees as part of the system, I think that minimum distance is going to be really impossible. If you can define it precisely enough, then genetically it is going to have to be easy enough to work with that its not going to meet the kind of requirements that it seems like the plant breeding industry has already laid out. And I think that the comments Mr. Greengrass made the first day in that what it ought to do, the plant protection ought to do, is to reward the creative inputs of plant breeders. It seems to me that that's a very good basis to underlie this. And if you're going to define minimum distance in terms of single gene traits or traits that are controlled very simply then that's not going to be the case.

James Wilcox (USDA, ARS, Purdue University): I don't see much of a problem in using qualitative traits to define differences among cultivars. I think the problem arises in using quantitatively inherited traits, and the production traits you refer to are virtually all quantitatively inherited traits. Now I think these are probably the kinds of traits where minimum distance becomes more critical.

Randall Nelson (USDA, ARS, University of Illinois): Let me add one thing onto that. I think that all that we've talked about here, in terms of trying to describe varieties, is very important and I didn't mean to minimize that. And I think that that's always going to be important, but I think to use those traits to define minimum distance is simply not adequate. Because, even regardless of what those traits are, you can have an extremely important economic trait; it may be controlled by a single gene and I don't think that anybody here is going to be satisfied with saying that a single, economically important trait, if its controlled by a single gene, is enough to distinguish varieties. It seems like the question that was raised is the technical determination of differences and the apportioning of differences between the varieties



that you have to.... There are two things that, sort of what we talked about yesterday, is that there are two things that we can get from descriptors. It's one thing to describe a variety so that one can always go back and determine that, yes, indeed, this is the variety that we have and that's important. But to use those kinds of traits to say that it is significantly different from this variety, that it should be allowed, seems to me to be something that the subcommittee working on here has already said, that that's not sufficient and we've already sort of worked that out I guess in an informal way among the US breeders. I think that it's appropriate to go ahead and move that into the system.

Kenneth Evans (PVPO): Well, for winter hardiness: I would say that we do differentiate varieties based on winter hardiness with data submitted from the applicant. For instance, in alfalfa, most US alfalfa varieties have been tested by universities or breeders and they can tell you relative winter hardiness. If they have a variety that'll grow in Arizona or California or someplace relatively non-winter hardy and contrast it to one that'll grow in Minnesota where it may require quite a little hardiness we would issue a certificate on that. Our law requires that we issue certificates on anything that is a clear difference. What that clear difference is, we have interpreted winter hardiness in alfalfa, at least, to be one of those things. If there's something that has a small range in winter hardiness and is difficult to test, we would probably have more trouble with that.

Barry Greengrass (UPOV): Could we have a French point of view from the panel?

Francois Blouet (INRA-GEVES): I can give you some ideas we are developing in France at an experimental stage. Of course, one observation is that the UPOV guidelines can bring some problems exactly like you describe. Because each characteristic is taken as itself, it has the same weight and, if this characteristic is governed by a single gene, then we can give plant breeders right to varieties which have been only selected for one trait and this trait can be very easy to select from a very good variety. So this can bring problems and this observation has led us to think of a hierarchy of characteristics based on the genetics of these characteristics. We think it could be a system to really protect the breeder. For instance, we could accept a difference between two varieties only based on one characteristic if it is with complex genetics. I mean, for instance, maturity date, height of plants. We know that these characteristics are not simple and not easy to select from an already existing variety. So this would be the first group of characteristics with what we call complex genetics. Another group would be characteristics governed by a few genes, like maybe hilum color in soya bean for instance, where we would allow at least two of those characteristics to assess distinctness. I mean one would not be sufficient but there should be at least two. And finally, the third group would be the characteristics with very simple genetics. One of our projects in maize, for instance, was to have three of those characteristics different to assess distinctness to be sure that there's really been breeding work and not only the cosmetic breeding. Finally,

I have to say that this system is thought to better use the breeder's description; that's very important. We think that this system could reduce the delay of studies in France maybe to one year. We think that it is also a better way of protecting the breeder.

Andre Heitz (UPOV): I just wanted to say that the reason I asked the first question was to comment this very question which is: whether any difference would be sufficient to, of course, establish distinctness, that is within the attitudes that's mathematical, but sufficient to grant rights to recognize the existence of an independent variety or whether there should be only a reduced number of such characteristics with then a need for defining those characteristics? We have heard a new line of thinking which exists in France, but also in the Netherlands, and I understand that there are two differing opinions now. There is one which says: anything that is a gene difference would be sufficient. I understand this is the practice in the Plant Variety Protection Office. The place where I work will not accept that.

Reid Palmer (USDA, ARS, Iowa State University): If I could just add a comment. At the present time, as you indicated, with PVP a single difference is enough to justify warrant of the PVP certificate but if more descriptors are needed, in other words two, three or four, then one is more or less obligated to go to molecular techniques in order to find these differences. So they may not have any economic value but at least they'll give you a greater magnitude of minimum distance. Instead of one, two or three, you can have four or five characteristics. This could perhaps be done presently with isozymes in soybean but if more distances are needed, or more descriptors for distances, one would probably go to restriction fragment length polymorphisms mainly because there you have more possibilities in terms in number of loci but also you have more alleles per locus. Rather than in the cases of soybeans with the W1 flower color locus you either have W1 or w1. If you have electrophoretic differences or RFLP differences you can have six, seven or more alleles per locus. So then it would be a lot more work to get these but at the same time you could be picking them up quite readily the more one looks at the molecular characteristics of soybean. So I think it depends really what the committee wants to do, how far the people will need to go in terms of molecular characterizations in order to get certificates.

Brian McBlain (OARDA-Ohio State University): I think that we should talk more about your difference in quality. How many characters you need to discern your variety depends on what you know about 'essentially derived'. For example, I happen to have a line in my program that's purple for only one parental site 31.7 for two parents that are unrelated to that A&B. Those genes sorted out and how its going to be different than that variety or other ones I'm still working on. Okay, I come over and included it in a cross and I may end up with one very small change relative to the parent type. There's always background. You can't avoid it in a whole different plant. Versus what if I'd used 31.7 as a parent and only had one small difference? And not so much 'essentially derived' by the way that we've discussed it here. But I

throw this out as a question from the standpoint of surely the plant breeder should be rewarded for developing a new variety and the other person with a prior variety, a variety in the market place, should bar that other one that's different in some plastic way. In other words, the French foreign hierarchical system may disallow such a divergent pedigree parent simply because it happened to look like another one. They may have only one or two traits rather than require three. I don't think that's fair. On the other hand is it fair that you select out of a biparental cross and come up that close and the only demonstrable difference is too close. That's a question that I have no answer to at this time.

John Batcha (Asgrow Seed Company): I hear a lot of good information and a lot of good ideas, problems, questions about the new molecular tools, about what the US law is. There's a lot of good ideas and a lot of good people in this room. The thing that comes to my mind is: how do you proceed on this? It's an important question. If we don't somehow proceed on this, some day they will end up forgotten, I don't know whether that's the best way to resolve this. If we as an industry, and I'm speaking bluntly, not just the seed companies but from the public workers, from the private workers, from people such as yourself, how should we go about addressing this question. I think it's real important that, before we leave in a half hour, an hour or so, where do we go from here? Do you have any thoughts on that? You've been involved in this longer than all of us in this room.

Barry Greengrass (UPOV): Well I think I would find that a very difficult question to respond to, if in fact there were not proposals in existence to modify the UPOV convention. Because the questions that we're discussing now are in fact no different from the questions that were being discussed back in the late 1950s when the experts who drafted the first Convention were doing their work. But we do fortunately have, I think, some new proposals that can radically address the situation. I said initially that the problem with the existing system is that we've asked the amount of difference there is in taxonomic terms between two varieties to do two things. The first taxonomic task under the Plant Variety Protection system is to so define the variety by using the distinctness, uniformity, stability criteria, but first and foremost distinctness, so that you can define what the right's going to attach to and that's the major task. Then the second task is to answer the question, what sort of plant breeding improvements should be protected? You then get involved in the merit question. Should you take into account merit? People have always felt that it shouldn't be just anything that can be protected but there's been probably historically some unclear thinking about what sort of things you should exclude and why you should exclude them. Here we meet the existing provisions of the UPOV Convention where the word 'important' was introduced to explain that it shouldn't be everything that you protect. It didn't thoroughly get the job done because the word 'important' has been interpreted to mean 'important for the purposes of establishing distinctness' so that the whole thing became circular and the underlying issues are not really addressed.



We're moving now towards a situation where it is possible to distinguish one variety from another very readily not only using some of the very small morphological characteristics that exist but also in the future using biochemical approaches. It was said on Thursday that if one wished, one could go so readily into existing varieties looking for tiny changes in the proteins and find off-types within them using biochemical tests. This would be very disturbing indeed because it would mean that almost any variety released by normal procedures could immediately be taken apart and the people who'd done the taking apart could immediately protect it. And that would be a major, significant threat to the viability of the whole system. So, one would be very worried about electrophoresis if there was no proposal to counter that threat. But, of course, we do now have this concept of 'essential derivation' or 'dependence', and we're then, in the future, going to ask this principle to deal with the aspect of minimum distance, which involves value judgements.

Should we protect a variety because it is an original piece of breeding? Perhaps we know we can distinguish it clearly and reliably and statistically, and we know that it constitutes an entity that we can attach rights to, but should we protect it? The way in which the essential derivation criterion approaches this is to say that you shouldn't protect a variety free of obligation to the breeder of the variety from which it is derived, if in fact the second variety has totally exploited the genetic structure of the first variety. We've got a lot of definition work and thinking to do in order to clarify the concept. We've had contributions in this meeting from various people on criteria that maybe we should begin to take into account: the objectives of the breeding process that was used, the intention, some people have talked about the cost and effort that was involved and so on and so forth. We need more criteria to help apply the new principle.

If you can imagine a situation where in the future varieties are protected if they're clearly distinguishable and, if you then have a well defined essential derivation principle in addition, then anybody who has spent ten or fifteen years breeding a variety will know that he will be given by the protection system, the tools to enable him to protect his end product. The system doesn't say it's a good end product or is better than some other end product, but the system simply enables him to protect it, even if it's in fact rather close to another variety. What the Plant Variety Protection system is doing is a bit like copyright in a book. It doesn't have to be a good book, but they can still get it copyrighted. Whether, in fact, it succeeds in the marketplace is another question. You have essentially the same situation with Plant Variety Protection, in that the work is protected. The system gives the breeder the tools to protect his variety. Whether it's going to be worthwhile or not depends on what then happens in the marketplace. The essential derivation system then addresses the question of whether the variety is free of obligation to the breeder of the variety from which the variety is essentially derived. If in fact the second variety is totally built upon the first one with some minor

change, then the first breeder should still have some rights in relation to the second variety without deciding at present what those rights should be. So that's the direction in which the UPOV revisions are moving and I think they do provide a framework within which we can actually address what were the really difficult aspects of minimum distance without having to use just the amount of distance between two varieties to try to decide if it's original enough to be freely protected. We hope to have a different set of criteria for that purpose. The only final remark I might add is: the minimum distance question doesn't go away with the advent of 'essential derivation'. We still have to decide whether 'A' is clearly distinguishable from 'B'. That, however, is a technical question for the experts, uncomplicated by whether it's an original variety or not, and the examiners present in the room can answer it without difficulty. Mr. Batcha asked what action is to be taken in relation to the minimum distance question and I hope my answer indicates the possible future directions. I think the general direction in which the revision is moving draws support from more or less everything that's been said in this meeting.

Grant Watson (Agriculture Canada): I'll ask this to one who's sort of familiar. Dr. Evans, the position of ASTA would be 'essentially derived'. In follow up to that, do you see that as a way to form an ad hoc committee to look at that? And would your legislation maybe be amended to adopt that kind of principle? Or where do you see going from here for the office?

Kenneth Evans (PVPO): Our legislation would have to be amended to adopt that. We have looked at the farmer's exemption. We have an advisory board and they have advised writing regulations to better define the farmer's exemption and our legal counsel has told us that our rule writing authority is very narrow and would not allow us to write that rule. And I'm sure that rule writing would not allow us to adopt this proposal either.

Jerry Peterson (ASTA): The American Seed Trade Association has had the subcommittee of our intellectual copyrights committee working on rewrite of the Plant Variety Protection Act and has defined two or three areas, particularly in the farmer's exemption, that need attention. Those proposed amendments have been presented to the Board in Washington at our annual meeting in June. We have since that time sought the support of other groups, seedsmen officials, and plant breeders, National Association of State Departments of Agriculture, and several state and regional organizations. And now I recently formed a committee to implement these proposed amendments to the Plant Variety Protection Act. And that committee is meeting in November to decide a course of action that will include proper endorsements in support, from commodity and other groups, to support our attempt to change the amendment. So ASTA has taken steps in that direction after the advisory counsel received information that the rules change was not possible. And we know there's no other alternative because of the tremendous abuse of this program of farmer's exemption. People on that committee are either people who have been strong all along or otherwise qualified to be on that panel.

Matter of fact I missed the bus trip yesterday because I had to go down to the ASTA office and be involved in the information that was going out to the committee members. It is being addressed.

Barry Greengrass (UPOV): Perhaps Dr. Schillinger would like to comment in a general way about the direction in which things are going. Do you feel that your minimum distance group in ASTA's requirements will be fulfilled by the sort of amendments that I've talked about being incorporated into a revised convention?

John Schillinger (Asgrow Seed Company): I see things starting to come together. I think there is, as we indicated the other day, that the breeders themselves have more or less dealt with the issue for one reason or another. But we as a group are feeling fairly confident that our rights and our investment in research could be protected through the 'essentially derived' amendment. So, from that standpoint, I think we're coming in together very nicely. I think also some of the information shared here in other forms, that even the descriptors and things like that, are being changed in order to solidify the ability to differentiate between varieties. I guess the question I would still propose is: in trying to redo the PVP Act we're going in with the farmer's exemption it sounds like. Is it wise to do one and then try to do the other later on? Dr. Hoerner, would it be a threat to some of the non-breeding type of biotech firms that would probably have other aspirations as far as using established germplasm or varieties to introduce their new technology?

Dennis Hoerner (Monsanto Company): I think there are two different issues. If someone utilizes a body of germplasm for their benefit, they have to accept the criteria on which that germplasm is protected. It really goes to the question that the gentleman asked in the back of the room. Direct transformation of a protected variety: is it or is it not preventable? I think the UPOV suggested changes make it pretty clear.

John Schillinger (Asgrow Seed Company): I guess my question was how much opposition would trying to introduce this type of legislation into the PVP Act create a non---

Dennis Hoerner (Monsanto Company): A non-conventional breeding precedence? I don't think you would get any resistance.

Barry Greengrass (UPOV): If I could perhaps contribute on that particular point? We have a meeting next month in Geneva when these revisions, proposals will be the subject of comment by non-governmental organizations worldwide including AIPPI, the International Chamber of Commerce, representatives of the patent profession, and so on. We've located most of the interest groups that are pursuing biotech and I think I can say without exception, all those bodies support the notion of the introduction of this 'essential derivation' concept and regard it as very progressive.



John Schillinger (Asgrow Seed Company): Jerry, to answer my first question about the two changes that we're looking upon at the PVP Act. How do you see that, timing-wise?

Jerry Peterson (ASTA): Our first formative strategy type meeting is in November and obviously we're going to be approaching other people, Ken Evans and other people of that type, to get their input on where we go from that point. We can embody within our resolution quite a bit of flexibility to add to the proposals at this point. It becomes a matter of strategy to determine whether or not you slay one dragon at a time or take on a whole army.

John Schillinger (Asgrow Seed Company): And I think another difficulty would be, I think as I mentioned to some of you already, that I think soybeans are quite advanced compared to the other species, and that we're dealing with that in other committees of ASTA on minimum distances. Some of the other species groups are really hung up on 'essentially derived' and some of these things for one reason or another. Even though we have a good feeling and a warm feeling here in the soybean group, I don't think that may be translated down into corn or sorghum or some of the other species.

Charles Caviness (University of Arkansas): Let me make just a couple of comments concerning my opinion along this line. One, I think I'm the elder statesman here of the public group, saying that what we can do I think is to win the public sector, that is the plant breeders, geneticists, in the public sector. That we need more information I think is very apparent on these traits. I mean we need to be able to, as Dr. Palmer showed to utilize the chemical traits that are highly inheritable. And this is really what's important, utilizing traits to differentiate types that have high inheritability, that is, they're not greatly affected by environment. We in the public sector I think are obligated, that's one of the charges that we have, to look into the basic information and find out more along this particular line whereas the private people are not as concerned in that particular area. What we can do, Randy and all of us, is maybe to emphasize, and it kind of stresses this to me in a meeting of this nature, that we need to look at the inheritance of these traits; to spend more time in that area, so this information would complement this minimum distance question that we're all concerned with. If we knew more about the traits it would be easier to define minimum distances. I think I'll go back with that in mind and maybe have some graduate students looking more into this particular area than I have in the past.

Thomas Salt (PVPO): I have a two-part question. The first part directed to Dr. Reid and that is the use of the tool itself. Knowing that genes jump and re-assort themselves in soybeans, is the use of RFLPs a useful tool at this point in applications for determining varieties? Secondly, the question has to be resolved whether its the total presence or absence of a band or the gradation in between. The second part of the question, I'll direct to Dr. Wilcox and Dr. Caviness and that is the scientific wisdom of what we're trying to do in terms of

the sensitivity of these tools applied to plant breeding. If we are going to use these characteristics to determine minimum distance, are the breeders willing to then clean up their varieties sufficient enough so that the sensitivity of these tools do not throw out errors? And is it wise to clean a variety to a genetically pure variety knowing that, if planted in monoculture for hundreds of thousands of acres, any new land race of disease is going to wipe through it?

Reid Palmer (USDA, ARS, Iowa State University): If one looks at maize, where the jumping genes or transposable elements are well known and well characterized, they have found that some of these families of transposable elements are present in essentially all of the open pollinated lines that have been looked at and in many of the inbreds. For example, one of the transposable element families is UQ, for ubiquitous, and that has proved to be true in a lot of the maize populations. Studies are underway now to determine, not so much the movement of these elements per se, that can be followed quite easily by using molecular probes, but what kind of variation that they might cause. And a lot of this variation that is being looked at has been quantitative traits. The facts just aren't in at the present time because these experiments take quite a while to do and fairly large populations. In the case of soybean, there is an endogenous transposable element system in soybean, the TGM1 family of elements. And those have been shown not to transpose. They're so defective that all you can do is find sequences of various sizes that presumably represent an active transposable element system. The one system that has been found in soybeans that is endogenous and is active, in fact was found initially at the flower color locus. And transposition from that locus has occurred and we have been assaying these, the end result or phenotypic result, the new mutants that occur. But at present there is no molecular probe for this active element and until a molecular probe is identified we really can't determine how many copies of the element are present in the soybean genome as either active copies or defective copies.

Charles Caviness (University of Arkansas): Changes do occur in soybean with this endogenous element. Now whether that would hinder using say RFLPs or isozymes as a technique or as a tool for classification of cultivars, I don't think so. Mainly I'm basing that upon what has been determined in maize. I think from another part of your question, was with RFLPs whether you look at, I believe, migration distances versus presence or absence, I don't see any problem there at all because in migrations you're usually looking at another active allele that just happens to have certain base pair changes or so, that affect in the case of electrophoresis net charge of the molecule. And so it migrates more or it migrates less. And so it's just a different allele but it's still an active form. In the case of the absence, we usually refer to those as nulls. And initially there was probably a fair bit of excitement thinking that these nulls perhaps are deletions and the product is not formed at all or is so defective that it's not functional. And this has been looked at a lot in soybeans for some of the nutritional components, particularly the bioxygenases, trypsin inhibitor and so on. Even when

one accumulates various nulls from different enzymes or proteins, it does not seem to have any detrimental effect upon the plant. So I would say based upon the evidence for isozymes and what's expected to occur for RFLPs, it would make no difference whether you have migratory differences or migration distances or whether you have presence or absence of these factors.

James Wilcox (USDA, ARS, Purdue University): I heard you ask me two questions. One was would I be willing to go through the steps necessary to absolutely purify a variety for protection.

Thomas Salt (PVPO): If we are going to rethink our criteria for uniformity of a variety.

James Wilcox (USDA, ARS, Purdue University): And I think my answer is simply would it be cost effective. If it's so expensive to do that, so that I couldn't see a return from plant variety protection justifying, I simply would not do it. If the varieties seem to merit that kind of expense, I would probably invest that kind of expense in it.

Thomas Salt (PVPO): What is your feeling towards the wisdom of going from a heterogeneous population down to trying to force a specific genotype.

James Wilcox (USDA, ARS, Purdue University): I see absolutely no problem with that at all. By nature soybean production in the US is not going to be absolutely uniform because of the sensitivity and adaptation of specific varieties to specific bands of latitude. Two other examples, the T cytoplasm in corn caused us a major problem one year only. After that the problem was essentially corrected, perhaps with more expense. The lack of homogeneity or the very heterogeneous American chestnut population did nothing to save that population from being wiped out as a timber species. So I don't see that as a major concern.

Charles Caviness (University of Arkansas): Well, just very briefly, of course as I stated the other day, that I firmly am convinced that we should have at least in my own opinion some heterogeneity within the varieties and I see no great problem with that at all if we describe the amount of variability that's in the population. And that's the plant protection group and all, they haven't really been too concerned if it's described. So describing the variability it really seems to me to satisfy the problem.

Unknown #1: If it's appropriate to change the subject a little bit. I want to just go back to some of the discussion we heard this morning dealing with the patent situation and so forth, and explore the area of patenting with utility patents, I guess, of genes that are in the germplasm collection versus unique combinations that have been derived by transformational or other kinds of techniques. I think that's of great concern not only to breeders in this country but also, and as was asked a little earlier, to other countries around the world because of



the fact that if a company or university or individual patents the gene, how does that affect the availability and the usefulness of that gene throughout the rest of the world.

Barry Greengrass (UPOV): Well I think we'd better treat this one fairly briefly. We could spend the whole afternoon discussing this, I'm sure.

Dennis Hoerner (Monsanto Company): All right, let me just say two things. One is that there's nothing to necessarily prohibit an attempt to patent a gene isolated from the plant genome. What we have to bear in mind though is to the extent that that plant gene was used in the prior art in the complex of a plant genome via routine plant breeding. The mere fact that you have a patent claim on the gene per se cannot take that away from the plant breeder. He can just not now take from your teaching and maybe use that compound per se to directly transform that single gene, if indeed it is an invention, into that variety. We can't lose sight of the fact that no claimed invention can remove from the public domain that which they have.

Unknown #1: So if it was in an introduction even though it wasn't used before by others it's still in that introduction and no one can take it away from the public.

Dennis Hoerner (Monsanto Company): I thought your question was more that the gene was being used in a complex sense but now they've isolated the particular gene and now your worry is I can no longer use it?

Unknown #1: Well, it could be that or it could be that it exists in the germplasm collection but we really haven't used it in the general sense. Well, take a disease resistance or something where you find resistance to a disease in the germplasm collection and patent that gene.

Dennis Hoerner (Monsanto Company): My immediate question is: is the person who's patenting the gene the first to recognize and appreciate that phenotypic trait?

Unknown #1: Yea. It's been in the collection for maybe twenty five years but nobody's actually worked on it for whatever reason. It's obvious that it's there and somebody can look for it but nobody did.

Dennis Hoerner (Monsanto Company): When you say it's obvious it was there, did the prior art appreciate its presence, the fact that this variety was more resistant, and appreciate that difference? There are a lot of things that are inherent. Microbes are inherent in the universe and yet there are ways to discover those, so to speak, and bring them to people's realization.

Unknown #1: And this would be similar only it is a gene instead of a complete organism.

Dennis Hoerner (Monsanto Company): I think that it really goes to: has that gene been exploited in a breeding sense? Well, then the breeders

never really had the benefit of that gene. That's my conclusion. It's present but they never captured the benefit of it. I think the more immediate concern is: can I have taken away from me, just because they can describe it better, that which I've exploited in the past. And in my opinion the answer is: they can limit how you can exploit it. You can't use the gene that they maybe now teach and use it to directly transform, but they cannot remove from your arsenal of tools that which you have already practiced because that is the prior art on which their invention is judged.

Barry Greengrass (UPOV): I think that particular statement will reassure the people on one particular point at least. Some guidance for UPOV could be drawn out of this meeting. Just one very brief point, would it seem that most people present in the meeting would like to see steps taken to advance the use of at least electrophoresis in relation to soybeans on a standardized basis? I have drawn that, I think, out of comments that people have made and some of the contributions. Because, if that is the case, I would like to take back to Switzerland with me a recommendation that UPOV should work upon an addendum to the existing soybean guideline to incorporate electrophoresis and develop standard recommendations for its use. Is that something that will be generally supported?

Unknown #2: Is the question should it be a requirement? Are you saying that should it be a requirement for....

Barry Greengrass (UPOV): Not so much a requirement, no. The guidelines specify characteristics and at the moment electrophoresis is not included. UPOV is beginning addenda to some of these guidelines on a species by species basis. I draw the conclusion that there's a lot of interest in the use of electrophoresis specifically for soybeans because of the comparative dearth of good characteristics. And if that is so then I think a recommendation from this group will be listened to by the UPOV technical committee. I gather there's nobody dissenting?

I think the time arrives to draw this workshop to a close. I think I've summarized what I'm taking home in a rather longish earlier contribution, so I won't duplicate that. I would however, simply like to take the opportunity to thank the Plant Variety Protection Office for organizing this UPOV workshop. I'd like to thank you all for attending. I'd like to thank Dr. Broome in particular for all the detail work which she has put into it. And I hope that this may be the first in a series of rather closer contacts between the European end of UPOV and persons interested in Plant Variety Protection here in the United States. So thank you very much indeed for having organized this meeting so successfully. I think it's the first one we've had and I believe it's off come very well. The audience that we've had has been the right audience; the people who understand the subject have been here. I certainly have been very pleased and very impressed with the outcome. Thank you very much.

Kenneth Evans (PVPO): I would like to thank all of our staff for their contributions. I would like to especially thank all the speakers for coming and giving very good advice or information on these things. I'd like to thank all of you from outside of the US for taking the time and effort to come here and the rest of you also. And I would invite you to come visit our Office this afternoon or some other time if you have time. I guess I will turn it over to Rose if she has any additional comments.

Rose Broome (PVPO): I'd like to echo what's been said. Thank you all for coming. Thank you so much for your inputs.

Andre Heitz (UPOV): I want to say that we always use the abusive language just because it's simple and convenient. One of the abuses which we have permitted during these days was to say on the one hand 'USA' and on the other hand 'UPOV'. I want to state that the USA is a member of UPOV and that it's a full member of UPOV. I am sure that this workshop has enabled those who always believed UPOV is the old continent plus the something else, that the rift between this country and the other one has been largely diminished.

Rose Broome (UPOV): Thank you very much. We take great heart in that statement and we look forward to many years of a much closer contact and cooperation with the old continent. Thanks very much. The workshop is closed.

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